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Chemical composition and larvicidal effects of essential oil of *Blumea martiniana* against *Anopheles anthropophagus*

Liang Zhu*, Ying-juan Tian

College of Food and Bioengineering, South China University of Technology, Guangzhou, Guangdong Province, China

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ABSTRACT

Objective: To determine chemical composition of the hydrodistilled essential oil of *Blumea martiniana*, and to evaluate its larvicidal activity against *Anopheles anthropophagus*, the natural vector of malaria. **Methods:** Chemical composition of essential oils extracted by steam distillation was investigated by gas chromatography and mass spectroscopy (GC-MS). Larvicidal activity of essential oil and its four main compounds was carried out by WHO method. Twenty larvae of early fourth-instar stage were used in the larvicidal assay and five replicates were maintained for each concentration. The 24 h LC_{50} and LC_{90} values were determined following probit analysis. **Results:** Totally 68 compounds corresponding to 98.55% of the total oil were identified and the major constituents of essential oil were: linalool(10.36%), germacrene D(9.09%), borneol(6.24%), γ-terpinene(5.38%). The oil and linalool, germacrene D, borneol, γ-terpinene exerted significant larvicidal activity with LC_{50} values of 46.86, 35.87, 44.61, 35.89, and 29.21 mg/L, respectively. It also showed a dose dependent effect on mortality. **Conclusions:** The essential oil of *Blumea martiniana* and its four major compounds may have potential for use in control of *Anopheles anthropophagus* larvae and could be useful in search of newer, safer and more effective natural compounds as larvicides.

1. Introduction

Human malaria is one of the leading causes of morbidity and mortality in the world. Despite the very significant regional decline in reported malaria cases in the 20th century, this disease still represents a significant public health problem in most developing countries in tropical and subtropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites[1]. It is estimated that the number of deaths due to malaria is 781 000 in 2009[2]. In China, malaria is still the leading cause of morbidity[3], there is evidence of a resurged of malaria since 2000[4], with more than 14 098 cases being notified in 2009.

Malaria is transmitted by the bites of *Anopheles* mosquitoes. In Asia, the mosquito *Anopheles anthropophagus (An. anthropophagus)* is the the major vector of malaria[5]. Eliminating the source of infection is an essential component for the control of mosquito-borne diseases[6]. During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of this disease vector by using synthetic insecticides has failed because the longterm harmful effects on non-target organisms and environment[7]. In addition, the continuous and indiscriminate use of conventional chemical insecticides has resulted in the development of physiological resistance[8,9].

Therefore, development of more efficient insect control materials and these materials, which have no ill effects on the non-target population, and are easily degradable are necessary[10]. The use of herbal products is one of the best alternatives for mosquito control. Several experiments have been reported on the larvicidal properties of plant essential oils against *Anopheles* mosquitoes. Essential oils extracted from *Azadirachta indica*[11] and leaves and rhizomes of *Curcuma longa*[12] demonstrated larvicidal activity against *Anopheles gambiae*. Essential oils of *Eucalyptus camaldulensis*[13], *Plectranthus amboinicus*[14], *Clausena anisata*[15], *Zanthoxylum armatum*[16], *Eucalyptus tereticornis*[17] and *Tagetes patula*[18] demonstrated larvicidal

*Corresponding author: Liang Zhu, College of Food and Bioengineering, South China University of Technology, 510641, Guangzhou, China.
Tel: +86 20 87113849
E-mail: zhuliang@scut.edu.cn
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activity against *Anopheles stephensi*.

The genus *Blumea*, classified in subtribe *Matricariinae* of the *Anthemideae*, comprises of about 80 species distributed in tropical and subtropical Asia, Africa, and Oceania. This genus is an important medicinal plant largely used as insecticide in traditional medicine. *Blumea martiniana (B. martiniana)*, an annual or perennial herb with light, camphor-like odor, is chiefly distributed in Southeast Asia, and has been used for the treatment of parasites and rheumatism in folklore medicine of Yunnan, China.

The objective of the present study was to examine the chemical composition of the hydrodistilled essential oil of *B. martiniana* by GC-MS, and to evaluate its larvicidal activity against *An. anthropophagus*. To the best of our knowledge, this is the first report on the chemical composition and larvicidal activity of the essential oil of *B. martiniana*.

2. Materials and methods

2.1. Plant material

B. martiniana, fresh aerial parts were collected in Shiwang Mountains, Guangxi Province, China in September 2010, and identified by Dr. Gong Xun. Voucher specimen (No 0046191) was deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

2.2. Isolation of the essential oil

The dried powder (500 g) of *B. martiniana* was chopped and subjected to hydrodistillation for 3 h using a clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 ℃ until further analysis.

2.3. GC-MS analysis

Quantitative and qualitative analysis of the essential oil was performed using a GC-MS 6890-5975 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5 MS fused silica capillary column (30 m ×0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 250 ℃ and 280 ℃, respectively. Essential oil solution (1 μL) in hexane was injected and analyzed with the column held initially at 40 ℃ for 1 min and then increased to 250 ℃ with a 3 ℃/min heating ramp and subsequently kept at 250 ℃ for 20 min. The Kovats indices were calculated for all volatile constituents using a homologous series of n-alkanes C_8-C_{25} on HP-5 MS column.The major components of oils were identified by co-injection with standards (wherever possible), confirmed with Kovats indices using the Wiley(V.7.0) and National Institute of Standards and Technology(NIST) V.2.0 GC-MS library. The relative concentration of each compound in essential oil was expressed as percentage by peak area normalization.

2.4. Mosquito larvicidal assay

The eggs of *An. anthropophagus* were from Centre for Disease Control and Prevention of Guangdong Province, China. Larvae were reared in 250 mL glass beaker and fed with Brewer's yeast/dog biscuit (1:3). Glass beaker with larvae were maintained at (27±2) ℃, (75±2)% relative humidity and photoperiod of 14:10 (L:D) h. Larvicidal activity of the essential oil and of the four main compounds (linalool, germacrene D, borneol and γ-terpinene which comprised 10.36%, 9.09%, 6.24% and 5.38%, respectively) isolated from the essential oil of *B. martiniana* were evaluated according to the standard procedures suggested by the World Health Organisation[19]. The essential oil and four main compounds were dissolved in 1 mL of ethanol solution and prepared into different concentrations (25, 50, 75, 100, 125 and 150 mg/L) using distilled water. Twenty larvae of early fourth-instar stage were used in the larvicidal assay and five replicates were maintained for each concentration. During this experiment no food was offered to the larvae. The larval mortality was calculated after 24 h of the exposure period.

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software using Statistical Package of Social Sciences (SPSS) 14.0 for windows, significance level was set at $P<0.05$.

3. Results

3.1. Chemical composition of the essential oil

The steam distillation of 500 g of dried plant material yielded 3.2 mL (0.64% v/w) essential oil. The oil was less dense than water and was a weak yellow colour. Totally 68 compounds were identified and accounted for 98.55% of total oil. The major chemical compounds of essential oil were: linalool(10.36%), germacrene D(9.09%), borneol(6.24%), γ-terpinene(5.38%), β-caryophyllene(4.76%), α-humulene(4.58%), allo-ocimene(4.35%), α-copaene(4.37%), allo-aromadendrene(4.32%), sabinene(4.17%), viridiflorene(4.06%). The percentage compositions of remaining 57 compounds ranged from 0.22% to 2.95%.

3.2. Larvicidal assays

The essential oils and its four major compounds were subjected to laboratory bioassay studies against *An. anthropophagus*. Essential oil induced (12.00±2.74)%, (52.00

±5.70)%, (86.00±5.47)%, (89.00±7.42)%, (98.00±2.74)% and (100.00±0.00)% larval mortality at the concentrations of 25, 50, 75, 100, 125 and 150 mg/L, respectively. The LC_{50} and LC_{90} values were 46.86 and 89.99 mg/L, respectively(χ^2=5.54, P<0.05). Among the four compounds, the most potent larvicidal compound was γ-terpinene, with LC_{50} and LC_{90} values of 29.21 and 63.10 mg/L, respectively(χ^2=3.31, P<0.05). Linalool had LC_{50} and LC_{90} values of 35.87 and 73.01 mg/L, respectively(χ^2=3.26, P<0.05). It was closely followed by borneol which showed LC_{50} and LC_{90} values of 35.89 and 85.00 mg/L, respectively(χ^2=4.32, P<0.05). The least potent among the four compounds was germacrene D, with LC_{50} and LC_{90} values of 44.61 and 96.18 mg/L, respectively(χ^2=3.56, P<0.05). In the control, 2.0% larval mortality was observed. It showed concentration-dependent effect on mortality (Table 1).

Table 1

Larvicidal activity of the four major compounds based on the concentration (%) from essential oil of *B. martiniana* against fourth instar larvae of *An. anthropophagus*.

Compounds	Concentration(mg/L)	24 h mortality(%)
Linalool	25	28.0±3.5
	50	70.0±4.5
	75	88.0±4.2
	100	98.0±2.7
	125	100.0±0.0
	150	100.0±0.0
Germacrene D	25	18.0±3.5
	50	57.0±6.5
	75	80.0±4.5
	100	88.0±3.5
	125	96.0±2.2
	150	100.0±0.0
Borneol	25	25.0±3.5
	50	67.0±5.5
	75	83.0±5.0
	100	92.0±4.5
	125	100.0±0.0
	150	100.0±0.0
γ-terpinene	25	4.2.0±4.2
	50	78.0±3.5
	75	93.0±2.7
	100	100.0±0.0
	125	100.0±0.0
	150	100.0±0.0

4. Discussion

Twelve monoterpene hydrocarbon fractions(18.30%), seventeen sesquiterpene hydrocarbon fractions(36.03%), fourteen oxygenated monoterpene fractions(26.38%), thirteen oxygenated sesquiterpenoid fractions(7.86%), and twelve others(9.98%) were identified in the essential oil. The major chemical compounds of *B. martiniana* essential oil were also found in essential oils of other genus *Blumea*. Sabinene and caryophyllene were major compounds of the essential oil from the aerial parts of *Blume perrottetiana*[20]. Borneol and caryophyllene were the dominant components in the essential oil of *Blume balsamifera* leaves[21]. Linalool, copaene, allo-ocimene, γ-terpinene and allo-aromadendrene were major compounds of the essential oil *Blume mollis(B. mollis)* leaves[22]. Germacrene-D, sabinene and γ-terpinene were main components of the essential oil of Blume brevipes[23]. β-Caryophyllene and α-humulene were main constituent of the essential oil of *Blume lacera* leaves[24]. The results indicate that the essential oil of *B. martiniana* share some relatively similar components with other species of *Blumea* and serve as chemosystematic markers of *B. martiniana*.

Mosquitoes in the larval stage are attractive targets for pesticides because it breeds in water and thus it is easy to deal with them in this habitat[25]. The *B. martiniana* essential oil and its four major compounds have excellent inhibitory effect against *An. anthropophagus* larvae and it is clearly illustrated that some isolated compounds including γ-terpinene, linalool, and borneol were more potent larvicides than the essential oil. It also shows a dose dependent effect on mortality, with increasing mortality of larvae as increasing concentrations of essential oil and compounds. In a earlier report[22], larvicidal activity of *B. mollis* oil against *Culex quinquefasciatus* with LC_{50} 71.71 ppm and LC_{90} 143.41 ppm. Some similar components were identified in this plant such as linalool, copaene, allo-ocimene, γ-terpinene and allo-aromadendrene.

The result of the present study were also comparable to the earlier reports on the larvicidal activities of the four major compounds, γ-terpinene, linalool, borneol and germacrene D. The LC_{50} values of γ-terpinene were 30.7 and 29.8 μg/mL against the larvae of *Anopheles aegypti(An. aegypti)* and *Anopheles albopictus*[26-31]. The LC_{50} values of linalool were 42.28, 38.64, and 35.17 ppm and of germacrene D were 21.28, 18.76, and 16.95 ppm against instar larvae of *Culex quinquefasciatus, An. aegypti* and *Anopheles stephensi* (*An. stephensi)*[15]. Two other study reported the LC_{50} values of linalool at 24 h were 155.73 μg/mL against fourth instar larvae of *Ochlerotatus caspius*[32] and the LC_{50} values of germacrene D were 63.6 and 59.5 μg/mL against the larvae of *An. aegypti* and *An. stephensi*[33]. The LC_{50} values of Borneol were 43.5 mg/L against the larvae of *An. aegypti*[34].

There is an urgent need to strength our arsenal against vector-borne mosquitoes. The present study has shown that the essential oil of *B. martiniana* and its four major compounds may have potential in control of *An. anthropophagus* larvae. The results could be useful in search of newer, safer and more effective natural compounds as larvicides. Further investigations of the action mode of the constituents, and of effects on non-target organisms and field evaluation are necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Greenwood BM, Fidock DA, Kyle DE, Kappe SHI, Alonso PL, Collins FH, et al. Malaria: progress, perils, and prospects for eradication. *J Clin Invest* 2008; **118**:1266-1276.

[2] World Health Organisation. *World malaria report 2010*. Geneva: WHO; 2010, p. 60-61.

[3] Tang LH. Progress in malaria control in China. *Chinese Med J* 2000; **113**: 89-92.

[4] Zhang WY, Wang LP, Fang LQ, Ma JQ, Xu YF, Jiang JF, et al. Spatial analysis of malaria in Anhui province, China. *Malaria J* 2008; **7**: 206.

[5] Geng YJ, Gao ST, Huang DN, Zhao YR, Liu JP, Li XH, et al. Differentially expressed genes between female and male adult *Anopheles anthropophagus*. *Parasitol Res* 2009; **105**: 843-851.

[6] Mabaso ML, Sharp B, Lengeler C. Historical review of malaria control in Southern Africa with emphasis on the use of indoor residual house-spraying. *Trop Med Int Health* 2004; **9**: 846-856.

[7] Wattal BL, Joshi GC, Das M. Role of agricultural insecticides in precipitating vector resistance. *J Comm Dis* 1981; **13**: 71-73.

[8] Severini C, Rom R, Marinucci M, Rajmond M. Mechanisms of insecticide resistance in field populations of *Culex pipiens* from Italy. *J Am Mosq Control Assoc* 1993; **9**: 164-168.

[9] Rao DR, Mani TR, Rajendran R, Joseph AS, Gajanana A, Reuben R. Development of high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. *J Am Mosq Control Assoc* 1995; **11**: 1-5.

[10]Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H, Jana M. Larvicidal activity of extracts from *Querus lusitania* var infectoria gals (oliv). *J Ethno Pharmacol* 2002; **79**: 261-263.

[11]Okumu FO, Knols BGJ, Fillinger U. Larvicidal effects of a neem *(Azadirachta indica)* oil formulation on the malaria vector *Anopheles gambiae*. *Malar J* 2007; **6**: 63.

[12]Ajaiyeoba EO, Sama W, Essien EE, Olayemi JO, Ekundayo O, Walker TM, et al. Larvicidal activity of turmerone-rich essential oils of *Curcuma longa* leaf and rhizome from nigeria on *Anopheles gambiae*. *Pharm Biol* 2008; **46**: 279-282.

[13]Medhi SM, Reza SDA, Mahnaz K, Reza AM, Abbas H, Fatemeh M, et al. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephens*. *Asian Pac J Trop Med* 2010; **3**: 841-845.

[14]Senthilkumar A, Venkatesalu V. Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against A*nopheles stephensi*: a malarial vector mosquito. *Parasitol Res* 2010; **107**: 1275-1278.

[15]Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2010; **3**: 874-877.

[16]Tiwary M, Naik SN, Tewaryb DK, Mittalc PK, Yadavc S. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *J Vector Borne Dis* 2007; **44**:198-204.

[17]Nathan SS. The use of Eucalyptus tereticornis Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour Technol* 2007; **98**:1856-1860.

[18]Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. *Bioresour Technol* 2005; **96**:1235-1240.

[19]World Health Organisation. *Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807*. Geneva: WHO; 1981.

[20]Owolabi MS, Lajideh L, Villanueva HE, Setzer WN. Essential oil composition and insecticidal activity of *Blumea perrottetiana* growing in Southwestern Nigeria. *Nat Prod Commun* 2010; **3**: 1135-1138.

[21]Bhuiyan MNI, Chowdhury JU, Begum J. Chemical Components in volatile oil from *Blumea balsamifera*(L.) DC. *Bangladesh J Botany* 2009; **38**:107-109.

[22]Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*. *Parasitol Res* 2008; **103**: 959-962.

[23]Mwangi JW, Achola KJ, Lwande W, Hassanali A, Laurent R. Constituents of the essential oil of *Blumea brevipes* (Oliv. & Hiern) Willd. *Flavour Frag J* 1994; **9**: 233-235.

[24]Laakso I, Seppanen-Laakso T, Hiltunen R, Ekundayo O. Composition of the essential oil of *Blumea lacera* DC.(Asteraceae) leaves from Nigeria. *Flavour Frag J* 1989; **4**:73-75.

[25]Amer A, Mehlhorn H. Larvicidal effects of varies essential oils against *Aedes*, *Anopheles*, and *Culex larvae* (Diptera, Culicidae). *Parasitol Res* 2006; **99**: 466-472.

[26]Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresour Technol* 2009; **100**: 452-456.

[27]Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43-48.

[28]Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124-129.

[29]Nikkon F, Habib MR, Saud ZA, Karim MR. *Tagetes erecta* Linn. and its mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pac J Trop Biomed* 2011; **1**(3): 186-188.

[30]Aziz AT, Dieng H, Hassan AA, Satho T, Miake F, Salmah MRC, et al. Insecticide susceptibility of the dengue vector *Aedes aegypti* (Diptera: Culicidae) in Makkah City, Saudi Arabia. *Asian Pac J Trop Dis* 2011; **1**(2): 94-99.

[31]Ravikumar H, Ramachandraswamy N, Puttaraju HP. Molecular strain typing of Wolbachia infection from Indian mosquitoes using wsp gene. *Asian Pac J Trop Dis* 2011; **1**(2): 106-109.

[32]Knio KM, Usta J, Dagher S, Zournajiam H, Kreydiyyeh S. Larvicidal activity of essential oil extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. *Bioresour Technol* 2008; **99**: 763-768.

[33]Kiran SR, Bhavani K, Devi PS, Rao BRR, Reddy KJ. Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi*. *Bioresour Technol* 2006; **97**: 2481-2484.

[34]Rajkumar S, Jebanesan A. Chemical composition and larvicidal activity of leaf essential oil from *Clausena dentata* (Willd) M. Roam. (Rutaceae) against the chikungunya vector, *Aedes aegypti* Linn. (Diptera: Culicidae). *J Asia-Pac Entomol* 2010; **13**: 107-109.