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ORIGINAL PAPER

Chemical composition and larvicidal activity of *Blumea densiflora* essential oils against *Anopheles anthropophagus*: a malarial vector mosquito

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Abstract *Blumea densiflora*, an edible and medicinal plant, is chiefly distributed in Southeast Asia and South Asia. Essential oils extracted by steam distillation from *B. densiflora* were investigated for their chemical composition and larvicidal activity against *Anopheles anthropophagus*, the primary vector of malaria in China and other East Asian countries. Totally, 46 compounds were identified by gas chromatography and mass spectroscopy. The major chemical compounds identified were borneol (11.43%), germacrene D (8.66%), β-caryophyllene (6.68%), γ-terpinene (4.35%), sabinene (4.34%), and β-bisabolene (4.24%). A series of concentrations of essential oil (that ranged from 6.25 to 150 ppm) were tested against *A. anthropophagus* fourth-instar larvae according to WHO recommendation. In general, larval mortality increased as concentration and exposure time increased, indicating a dose-dependent effect, and high insecticidal activity showed that 100% mortality occurred within 6 h at 150 ppm, 10 h at 100 ppm, 30 h at 50 ppm, and 30 h at 25 ppm essential oil concentration. The LC_{50} values were 22.32 (after 12 h) and 10.55 ppm (after 24 h), and the LC_{90} values were 54.04 (after 12 h) and 33.56 ppm (after 24 h). Pylarvex, the reference standard, had better larvicidal activity, causing 100% mortality within 2 h at 150 ppm and within 6 h at 6.25 ppm. The results clearly reveal that the essential oil of *B. densiflora* served as a potential, eco-friendly mosquito larvicide against the malarial vector mosquito *A. anthropophagus*.

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Introduction

Mosquitoes are blood-feeding insects and serve as the most important vectors for spreading human diseases, such as malaria, yellow fever, dengue fever, and filariasis (James 1992). Human malaria is a complex disease, and its incidence is a function of the interaction between the *Anopheles* mosquito vector, the parasite (*Plasmodium* species), humans, and the environment. The disease is considered the most important vector-borne disease worldwide, primarily affects poor populations, and is still a serious public health problem of most developing countries in tropical and subtropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites (Greenwood et al. 2008). According to the World Health Organization (WHO 2010), there may be over 225 million malaria infections in tropical and subtropical countries for 2009, resulting in 781,000 deaths. In China, the incidence of malaria decreased dramatically from the 1950s to the 1990s. However, there is evidence of a resurge of malaria since 2000, with more than 14,098 cases being notified in China in 2009.

Malaria is transmitted by the bites of *Anopheles* mosquitoes. Most species of *Anopheles* in temperate East Asia are part of the *Hyrcanus* group of the subgenus *Anopheles*. The mosquito *Anopheles anthropophagus* is the primary vector of malaria in China and other East Asian countries (Liu 1990).

Due to the absence of effective vaccines against malaria, the control of a growing mosquito population is an important public health concern around the world. Chemical control is an obvious method used extensively in daily life for the control of mosquitoes. Many synthetic organic chemical insecticides such as organochlorine, organophosphorus, carbamates, pyrethrins, and pyrethroids have been developed

and employed in the field. However, the management of this disease vector by using synthetic insecticides has failed because they not only affect the non-target population but can also constantly increase mosquito resistance to the insecticide (Wattal et al. 1981). In this regard, the emphasis to control the mosquito populations has steadily shifted from the use of conventional synthetic insecticides towards more efficient insect control materials which do not have any ill effects on the non-target population, and are easily degradable are always sought (Redwane et al. 2002). For this purpose, chemicals derived from plant material have been increasingly used for mosquito control because of their efficacy and documented environmentally safe, degradable, and non-toxic effects on non-target organisms (Koliopoulos et al. 2010; Conti et al. 2010).

Essential oils from plants are widely used in the prevention and treatment of human illnesses and have been suggested as alternative sources of mosquito larval control agents. In fact, several experiments have been reported on the larvicidal properties of essential oils against *Anopheles* mosquitoes. Essential oils extracted from *Plectranthus amboinicus* (Senthilkumar and Venkatesalu 2010), *Eucalyptus camaldulensis* (Medhi et al. 2010), *Eucalyptus tereticornis* (Nathan 2007), *Zanthoxylum armatum* (Tiwary et al. 2007), *Tagetes patula* (Dharmagadda et al. 2005), and the leaves of *Clausena anisata* (Govindarajan 2010) demonstrated larvicidal activity against *Anopheles stephensi*. Essential oils extracted from leaves and rhizomes of *Curcuma longa* (Ajaiyeoba et al. 2008) and *Azadirachta indica* (Okumu et al. 2007) demonstrated larvicidal activity against *Anopheles gambiae*.

The genus *Blumea*, classified in subtribe Matricariinae of the Anthemideae, comprises about 80 species distributed in tropical and subtropical Asia, Africa, and Oceania. This genus is an important medicinal plant largely used as an insecticide in traditional medicine, and the pharmacological study of the essential oils of several species of the genus have been examined (Gupta et al. 1977; Senthilkumar et al. 2008; Owolabi et al. 2010).

Blumea densiflora is a perennial herb or subshrub, 1–3 m high, with erect, stout stems and soft glandular hairs. The leaves are nearly sessile, elliptic, pinnatifid, and irregularly toothed. The numerous capitula are yellow in color, bracts are narrowing and acuminate, and achenes are terete (Fig. 1). *B. densiflora* is chiefly distributed in Southeast Asia and South Asia and has long been collected both as an edible and medicinal plant for malaria, fever, enteritis, and high blood pressure.

This plant is used to drive away mosquitoes by burning, and its juice used to prevent mosquito bites in South China because of its light, borneol-like odor. Therefore, the aim of this study was to determine the chemical composition of the hydrodistilled essential oil of *B. densiflora* by gas chromatography–mass spectrometry (GC–MS) and to evaluate its larvicidal activity against *A. 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. densiflora*.

Fig. 1 *B. densiflora*

Materials and methods

Plant material

B. densiflora fresh aerial parts were collected in Gaoligong Mountains, Yunnan Province, China, in June 2008 and identified by Dr. Gong Xun. A voucher specimen (no. 0046091) was deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Isolation of the essential oil

The dried powder (500 g) of *B. densiflora* 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°C until further analysis.

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 temperatures were set at 250 and 280°C, respectively. Essential oil solution (1 μL) in hexane was injected and

analyzed with the column held initially at 40°C for 1 min and then increased to 250°C with a 3°C/min heating ramp and subsequently kept at 250°C 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 the 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 V.2.0 GC–MS library. The relative concentration of each compound in the essential oil was expressed as percentage by peak area normalization.

Mosquito larvicidal assay

The eggs of *A. anthropophagus* were received from the Centre for Disease Control and Prevention of Guangdong Province, China. Larvae were reared in a 250-ml glass beaker and fed with Brewer's yeast/dog biscuit (1:3). The glass beaker with the larvae was maintained at 27±2°C, 75±2% relative humidity and photoperiod of 14:10 h (L/D). The larval mortality bioassays were carried out according to the standard procedures suggested by the World Health Organization (1981). The essential oil was dissolved in 1 ml of DMSO solution and prepared into different concentrations (6.25, 12.5, 25, 50, 100, and 150 ppm) using distilled water. For comparison, commercial larvicide Pylarvex® (Pyrethrum Board of Kenya) was used as positive control. Twenty larvae of the 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 12 and 24 h of the exposure period. The lethal concentrations LC_{50} and LC_{90}, and their 95% confidence intervals, and the upper and lower confidence levels were calculated using profit analysis (SPSS, version 14.0).

Results and discussion

Chemical composition of the essential oil

Gaoligong Mountains, located at the linking point among China, Indian sub-continent, and China–Indian Peninsula, is one of the richest area of the world in terms of having a substantial number of different medicinal plant species grown in tropic and subtropic ecological conditions. The investigation of larvicidal properties of these plants has brought the opportunity of producing a natural-based, degradable, and environment friendly new source that could replace the synthetic insecticide. To the best of our knowledge, the essential oil compositions of *B. densiflora*

Table 1 Chemical composition of the essential oil of *B. densiflora*

Peak no.	RI[a]	Components	% RA[b]	Identification methods[c]
1	902	Heptanal	0.54	MS, RI
2	928	α-Thujene	0.52	MS, RI
3	936	α-Pinene	2.74	MS, RI
4	949	Camphene	2.13	MS, RI
5	973	Sabinene	4.34	MS, RI, Co
6	978	β-Pinene	1.23	MS, RI
7	1,010	a-Phellandrene	1.65	MS, RI
8	1,012	3-Carene	1.18	MS, RI
9	1,017	*p*-Cymene	2.12	MS, RI
10	1,022	*o*-Cymene	2.20	MS, RI
11	1,030	*d*-Limonene	3.38	MS, RI, Co
12	1,032	β-Phellandrene	1.22	MS, RI
13	1,040	(*Z*)-β-Ocimene	1.85	MS, RI
14	1,051	(*E*)-β-Ocimene	1.35	MS, RI
15	1,059	γ-Terpinene	4.35	MS, RI, Co
16	1,099	Linalool	2.56	MS, RI
17	1,139	*E*-Pinocarveol	0.47	MS, RI
18	1,143	Camphor	2.35	MS, RI
19	1,160	Isoborneol	2.67	MS, RI
20	1,168	Borneol	11.43	MS, RI, Co
21	1,170	Lavandulol	0.74	MS, RI
22	1,191	α-Terpineol	1.06	MS, RI
23	1,232	Citronellol	0.89	MS, RI
24	1,255	Geraniol	0.64	MS, RI
25	1,286	Bornyl acetate	0.69	MS, RI
26	1,357	Eugenol	2.37	MS, RI
27	1,384	β-Bourbonene	0.68	MS, RI
28	1,390	β-Elemene	1.05	MS, RI
29	1,408	Methyl eugenol	0.58	MS, RI
30	1,418	β-Caryophyllene	6.68	MS, RI, Co
31	1,436	β-Gurjunene	2.21	MS, RI
32	1,442	*E*-α-Bergamotene	1.25	MS, RI
33	1,454	α-Humulene	2.42	MS, RI, Co
34	1,460	*Allo*-aromadendrene	1.46	MS, RI
35	1,486	Germacrene D	8.66	MS, RI,Co
36	1,506	β-Bisabolene	4.24	MS, RI, Co
37	1,531	Cadina-1,4-diene	0.67	MS, RI
38	1,562	*E*-Nerolidol	1.28	MS, RI
39	1,567	Ledol	0.95	MS, RI
40	1,578	Caryophyllene oxide	1.56	MS, RI
41	1,598	Guaiol	0.58	MS, RI
42	1,611	Zingiberenol	1.24	MS, RI
43	1,619	10-epi-γ-Eudesmol	1.35	MS, RI
44	1,624	7-epi-γ-Eudesmol	2.24	MS, RI
45	1,654	α-Eudesmol	1.50	MS, RI
46	1,971	*n*-Hexadecanoic acid	1.36	MS, RI
		Total identified (%)	98.63	
		Monoterpene hydrocarbons	25.94	
		Monoterpenoids	25.18	
		Sesquiterpene hydrocarbons	29.32	
		Sesquiterpenoids	10.70	
		Others	7.49	

RI retention index relative to *n*-alkanes on HP-5 MS capillary column, *RA* relative area (peak area relative to the total peak area), *MS* mass spectrum, *Co* co-injection with authentic compound

have not been reported before, and these results were first evidenced on the composition of this endemic species.

The steam distillation of 500 g of dried plant material yielded 2.9 ml (0.58% *v*/*w*) light yellow oil with a distinct smell. Table 1 shows the chemical composition of the essential oil analyzed by GC–MS. Totally, 46 compounds were identified and account for about 98.63% of the total oil. Among these, the monoterpene hydrocarbon fraction was 25.94% of the oil, while the oxygenated monoterpene fraction was 25.18%. The sesquiterpene hydrocarbon fraction was 29.32%, and the oxygenated sesquiterpenoid fraction was 10.70% in the oil. The major chemical compounds were: borneol (11.43%), germacrene D (8.66%), β-caryophyllene (6.68%), γ-terpinene (4.35%), sabinene (4.34%), and β-bisabolene (4.24%).

In several earlier reports, the essential oils of the genus *Blumea* have been examined. The essential oil from the aerial parts of *Blumea perrottetiana* was dominated by 2,5-dimethoxy-*p*-cymene (30.0%) and 1,8-cineole (11.0%) with lesser amounts of sabinene (8.1%), delta-cadinene (5.3%), and (*E*)-caryophyllene (3.9%) (Owolabi et al. 2010). The dominant components in the essential oil from *Blumea balsamifera* leaves were borneol (33.22%), caryophyllene (8.24%), ledol (7.12%), tetracyclo[6,3,2,0,(2.5).0(1,8)tridecan-9-ol, 4,4-dimethyl] (5.18%), phytol (4.63%), caryophyllene oxide (4.07%), guaiol (3.44%), thujopsene-13 (4.42%), dimethoxy-durene (3.59%), and γ-eudesmol (3.18%) (Bhuiyan et al. 2009). The major chemical compounds of the essential oil from the leaves of *Blumea mollis* identified were linalool (19.43%), γ-elemene (12.19%), copaene (10.93%), estragole (10.81%), *allo*-ocimene (10.03%), γ-terpinene (8.28%), and *allo*-aromadendrene (7.44%) (Senthilkumar et al. 2008). The main components of the essential oil of *Blumea brevipes* were terpinen-4-ol (27.6%), germacrene D (15.4%), sabinene (8.0%), and γ-terpinene (5.5%) (Mwangi et al. 1994). The main constituent of the essential oil of *Blumea lanceolaria* was methyl thymol (Dung et al. 1991). The main constituent of the essential oil of *Blumea lacera* leaves was thymoquinol di-mether, β-caryophyllene, α-humulene, and *E*-β-farnesene (Laakso et al. 1989). The results indicate that the essential oil of *B. densiflora* shares some relatively similar components with that of other species of *Blumea* and serve as chemo-systematic markers of *B. densiflora*.

Larvicidal assays

Amer and Mehlhorn (2006a) clearly stated that mosquitoes in the larval stage are attractive targets for pesticides because they breed in water, and thus, it is easy to deal with them in this habitat. Thus, many researchers were intrigued to exploit essential oils with larvicidal efficacy as a potential source against medically important vectors (Trabousli et al. 2005; Amer and Mehlhorn 2006b, c; Rahuman et al. 2008; Rahuman and Venkatesan 2008).

In the present study, a series of concentrations of *B. densiflora* essential oil from 6.25 to 150 ppm and commercial Pylarvex were tested for their larvicidal activity against *A. anthropophagus* fourth-instar larvae. Table 2 displays the results on percent mortality of larvae of *A.*

Table 2 Percent mortality (mean ± SD) of larvae of *A. anthropophagus* at different concentrations of essential oil of *B. densiflora* and at different time intervals

Time (h)	Concentration (ppm)								
	6.25	12.5	25	50	100	150	P 6.25	P 150	Control
1	0±0.0	0±0.0	8±4.5	19±4.2	33±2.7	52±4.5	43±5.5	88±2.7	0
2	8±2.7	10±3.5	26±4.2	37±4.5	52±2.7	82±7.6	53±5.5	100±0.0	0
4	11±5.5	12±4.5	39±8.2	44±2.2	72±4.5	96±4.2	86±4.5		0
6	15±3.5	16±4.2	45±7.1	50±3.5	83±2.7	100±0.0	100±0.0		0
8	16±4.2	21±2.2	48±2.7	55±6.2	90±3.5				0
10	19±2.2	26±6.5	54±6.5	61±4.2	100±0.0				0
12	24±4.2	32±4.5	62±2.7	84±5.0					0
18	31±5.5	38±2.7	81±4.2	92±7.6					0
24	36±4.2	54±8.2	86±7.6	97±4.5					0
30	48±4.5	82±5.7	100±0.0	100±0.0					1±2.2
36	76±6.5	97±4.5							1±2.2
42	86±6.5								1±2.2
48	91±4.2								1±2.2

P commercial Pylarvex

Table 3 The effect of essential oil of *B. densiflora* against larvae of *Anopheles anthropophagus*

Exposure time (h)	LC_{50} (95% FL)	LC_{90} (95% FL)	Regression equation	Chi-square
12	22.32 (18.98–25.68)	54.04 (47.85–62.98)	y=−0.901+0.040x	3.631
24	10.55 (7.28–13.21)	33.56 (29.46–39.70)	y=−0.588+0.056x	4.354

LC_{50} lethal concentration (ppm) at which 50% of the larvae showed mortality, *LC_{90}* lethal concentration (ppm) at which 90% of the larvae showed mortality, *x* log concentration, *y* percentage mortality

anthropophagus with increase in essential oil and Pylarvex concentration and with respect to different time periods. The tested essential oils showed high insecticidal activity that 100% mortality occurred within 6 h at 150 ppm and 10 h at 100 ppm essential oil concentration. At higher concentrations (50 to 150 ppm), the larvae showed restless movement, then sank to the bottom of the beakers with abnormal wagging and died slowly. A positive correlation was observed between the essential oil concentration and the larvicidal activity, the rate of mortality being directly proportional to the concentration, indicating a dose-dependent effect on mortality. Pylarvex, the reference standard, had better larvicidal activity; 150 ppm caused 100% mortality of *A. anthropophagus* within the first 2 h. Table 3 summarizes the LC_{50} and LC_{90} values after 12 and 24 h of exposure period. The LC_{50} values of *B. densiflora* oil were 22.32 (after 12 h) and 10.55 ppm (after 24 h), and the LC_{90} values were 54.04 (after 12 h) and 33.56 ppm (after 24 h).

Several studies have reported insecticidal and acaricidal activities of essential oils from the genus *Blumea.* The essential oil from the leaves of *B. mollis* had a significant toxic effect against early fourth-instar larvae of *Culex quinquefasciatus* (Senthilkumar et al. 2008). The essential oil from the aerial parts of *B. perrottetiana* demonstrated notable insecticidal activity against *Tribolium castaneum* (Owolabi et al. 2010). The essential oils of *B. lacera* and *B. malcomii* increased the insecticidal activity of pyrethrum against *Musca domestica* (Gupta et al. 1977). Table 4 compares our results with those obtained by other studies.

The activity against *A. anthropophagus* may be attributed to the presence of the major chemical compounds of oil such as borneol, germacrene D, β-caryophyllene, sabinene, and γ-terpinene. Rajkumar and Jebanesan (2010) demonstrated that borneol and sabinene, which were the major components of *Clausena dentata* essential oil, showed larvicidal activity against *Aedes aegypti*. Germacrene D has larvicidal activity against *A. aegypti* and *A. stephensi* (Kiran et al. 2006). The larvicidal activity of γ-terpinene against *A. aegypti* and *Aedes albopictus* was also investigated (Cheng et al. 2009).

On the other hand, there is some evidence indicating that essential oils often proved to be more effective than their components, indicating the phenomenon of synergy (Don-Pedro 1999). Therefore, the multi-component system of *B. densiflora* essential oil that was composed mainly of terpenes may be responsible for the larvicidal activity.

It is an urgent need to strengthen our arsenal against vector-borne mosquitoes. The present study demonstrates that the essential oil extracted from *B. densiflora* has strong larvicide potential against *A. anthropophagus* and is an

Table 4 Summary of insecticidal activity and major composition of essential oils from *Blumea* species

Source and reference	Insecticidal activity	Major composition
B. mollis (Senthilkumar et al. 2008)	Significant toxic effect against early fourth-instar larvae of *Culex quinquefasciatus* (LC_{50}=71.71 ppm and LC_{90}=143.41 ppm, 24 h)	Linalool (19.43%), γ-elemene (12.19%), copaene (10.93%), estragole (10.81%), *allo*-ocimene (10.03%), γ-terpinene (8.28%), *allo*-aromadendrene (7.44%)
aerial parts of *B. perrottetiana (*Owolabi et al. 2010*)*	Notable insecticidal activity against the red flour beetle, *Tribolium castaneum*	2,5-Dimethoxy-*p*-cymene (30.0%), 1,8-cineole (11.0%), sabinene (8.1%), δ-cadinene (5.3%), β-caryophyllene (3.9%)
B. lacera and *B. Malcomii (*Gupta et al., 1977*)*	The LD_{50} of pyrethrum was decreased from 0.0115 g/fly to 0.0033 and 0.0060 g/fly by the essential oils of *B. lacera* and *B. malcomii*, respectively.	No investigation
B. Densiflora	Toxic effect against fourth-instar larvae of *Anopheles anthropophagus* (LC_{50}=22.32 ppm and LC_{90}=54.04 ppm, 12 h; LC_{50}=10.55 ppm and LC_{90}=33.56 ppm, 24 h)	Borneol (11.43%), germacrene D (8.66%), β-caryophyllene (6.68%), γ-terpinene (4.35%), sabinene (4.34%), β-bisabolene (4.24%)

environmentally safe, inexpensive, and practical mosquito larvicide to control/reduce the population of malarial vector mosquitoes, especially when the tested herbs are commonly grown and used in Southeast Asia and South Asia where malaria is a serious problem. However, field evaluation and further investigations on the effects on non-target organisms are necessary.

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References

Ajaiyeoba EO, Sama W, Essien EE, Olayemi JO, Ekundayo O, Walker TM, Setzer WN (2008) Larvicidal activity of turmerone-rich essential oils of *Curcuma longa* leaf and rhizome from Nigeria on *Anopheles gambiae*. Pharm Biol 46:279–282

Amer A, Mehlhorn H (2006a) Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). Parasitol Res 99:466–472

Amer A, Mehlhorn H (2006b) Repellency effect of forty-one essential oils against *Aedes, Anopheles*, and *Culex* mosquitoes. Parasitol Res 99:478–490

Amer A, Mehlhorn H (2006c) The sensilla of *Aedes* and *Anopheles* mosquitoes and their importance in repellency. Parasitol Res 99:491–499

Bhuiyan MNI, Chowdhury JU, Begum J (2009) Chemical components in volatile oil from *Blumea balsamifera* (L.) DC. Bangladesh J Botany 38:107–109

Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST (2009) Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. Bioresour Technol 100(1):452–456

Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L (2010) Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 107:1455–1461

Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P (2005) Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. Bioresour Technol 96(11):1235–1240

Don-Pedro KN (1999) Investigation of single and joint fumigant insecticidal action of citrus peel oil components. Pestic Sci 46:79–84

Dung NX, Loi DT, Hung DT, Leclercq PA (1991) Chemical composition of the oil of *Blumea lanceolaria* (Roxb.) Druce from Vietnam. J Essent Oil Res 3:285–286

Govindarajan M (2010) 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 3:874–877

Greenwood BM, Fidock DA, Kyle DE, Kappe SHI, Alonso PL, Collins FH, Duffy PE (2008) Malaria: progress, perils, and prospects for eradication. J Clin Invest 118:1266–1276

Gupta SC, Khanolkar UM, Koul O, Saxena BP (1977) Pyrethrin synergistic activity by the essential oils of a few *Blumea* species. Curr Sci 46:304–305

James AA (1992) Mosquito molecular genetics: the hands that feed bite back. Science 257(5066):37–38

Kiran SR, Bhavani K, Devi PS, Rao BRR, Reddy KJ (2006) Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi*. Bioresour Technol 97(18):2481–2484

Koliopoulos G, Pitarokili D, Kioulos E, Michaelakis A, Tzakou O (2010) Chemical composition and larvicidal evaluation of *Mentha, Salvia*, and *Melissa* essential oils against the West Nile virus mosquito *Culex pipiens*. Parasitol Res 107:327–335

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

Liu CF (1990) Comparative studies on the role of *Anopheles anthropophagus* and *Anopheles sinensis* in malaria transmission in China. Chinese J Epidemiol 11(6):360–363

Medhi SM, Reza SDA, Mahnaz K, Reza AM, Abbas H, Fatemeh M, Hassan V (2010) Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephens*. Asian Pac J Trop Med 3:841–845

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

Nathan SS (2007) 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 98(9):1856–1860

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

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

Rajkumar S, Jebanesan A (2010) 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 13:107–109

Rahuman AA, Venkatesan P (2008) Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. Parasitol Res 103:133–139

Rahuman AA, Venkatesan P, Gopalakrishnan G (2008) Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitol Res 103:1383–1390

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

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

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

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

Trabousli AF, El-Haj S, Tueni M, Taoubi K, Nader NA, Mrad A (2005) Repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens* molestus (Diptera: Culicidae). Pest Manag Sci 6:597–604

Wattal BL, Joshi GC, Das M (1981) Role of agricultural insecticides in precipitating vector resistance. J Comm Dis 13:71–73

World Health Organisation (1981) Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO, Geneva, pp 1–6

World Health Organisation (2010) World Malaria Report 2010. WHO, Geneva, pp 60–61