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Larvicidal Activity of Turmerone-Rich Essential Oils of *Curcuma longa* Leaf and Rhizome from Nigeria on *Anopheles gambiae*

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Abstract

The essential oils from the leaves and rhizomes of *Curcuma longa* L. (Zingiberaceae) were subjected to larvicidal toxicity studies on *Anopheles gambiae*, the malaria vector. The leaf essential oils were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry, respectively. The rhizome oil was much more toxic to the mosquito larvae, exhibiting 100% mortality at 0.125 mg/mL with an LC_{50} of 0.017 mg/mL. The leaf had absolute mortality at 0.500 mg/mL with an LC_{50} of 0.029 mg/mL. The observed toxicities were also found to be concentration dependent. The oils were found to be composed mainly of turmerones, with the major components in the leaf volatile oil being ar-turmerone (63.4%), α-turmerone (13.7%), and β-turmerone (12.6%). ar-Turmerone (44.4%), β-turmerone (26.5%), and α-turmerone (20.8% were the main components in the rhizome. Both oils displayed overwhelming activities compared with the reference compound N,N-Diethyl-m-toluamid (DEET) which had an LC_{50} value of 1.09 mg/mL. The turmerone composition, especially the combination of α-turmerone and β-turmerome constituents in the oils, may be responsible for the observed larvicidal toxicities of both essential oils. The essential oils from the leaf and rhizome of this plant may find use as a source of malaria vector control agents.

Keywords: *Anopheles gambiae, Curcuma longa,* essential oils, larvicidal activity, mosquitoes, turmerone.

Introduction

Malaria remains one of the devastating diseases transmitted by mosquitoes and currently represents a great health problem in tropical and subtropical climates, with no part of the world immune to this risk (Fradin & Day, 2002). Globally, more than 2 billion people live in areas threatened by malaria. The morbidity and mortality associated with the disease is mostly expressed in sub-Saharan Africa (Bremen et al., 2001; WHO, 2000). Annually, there is an estimated 2 million deaths from malaria. This burden is highest in children under 5 years of age (WHO, 1998). One of the approaches for control of malaria is the interruption of its transmission eliminating the malaria vector. There has been serious concern about the use of chemical-based mosquitocides in the recent past. The extensive use of these synthetic organic insecticides during the past five decades has resulted in environmental hazards and also in the development of physiologic resistance in major vector species. This has necessitated the need for discovery and development of environmentally safe, biodegradable, economically viable and indigenous methods for vector control. Some herbal products such as nicotine obtained from tobacco leaves, *Nicotiana tabacum*, anabasine and lupinine, two alkaloids extracted from Russian weed *Anabasis aphylla*, rotenone from *Derris eliptica,* and pyrethrums from *Chrysanthemum cinererifolium* flowers have been used as natural insecticides even before the discovery of synthetic organic insecticides (Ansari & Razdan, 1994). Several plant oils and extracts have and are being tested for larvicidal property,

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particularly on the malaria vector (Ansari & Razdan, 1994, 1995; Ansari et al., 1999).

Curcuma longa L. (syn. *C. domestica* Vahl.) is a perennial rhizomatous herb of the family Zingiberaceae. The rhizome is the source of turmeric, which has use as a condiment and coloring agent in medicines, confectionery and curry powder (Abbiw, 1990). Turmeric has a long traditional use in the Chinese and Ajurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, and menstrual difficulties (Thorn Research, Inc., 2001). Constituents of the leaf, rhizome, and flowers have been studied extensively (Banal et al., 2002; Chane-Ming et al., 2002; Oguntimein et al., 1990; Riaz et al., 2000; Sakuntala et al., 2002). The rhizome essential oil is reported to exhibit antimicrobial insecticidal, larvicidal, repellency, and antioxidant activities (Martins et al., 2001; Pandian & Kathiresan, 2002; Rambir et al., 2002, Sacchetti, 2005; Tawatsin et al., 2001). The leaf oil has also been reported to exhibit fumigant toxicity against stored-products beetles (Tripithi et al., 2002). This paper reports the chemical constitution of the essential oils of the leaf and rhizome of *C. longa* and their toxicity to the larvae of *Anopheles gambiae* mosquitoes.

Materials and Methods

Plant material

Fully grown leaves and rhizomes of *C. longa* were collected from plants cultivated in Mbiaso village, Ikot Ekpene Local Government Area of Akwa Ibom State, Nigeria, in October 2004. Plant materials were authenticated by Mr. F. Usang of the Forest Research Institute of Nigeria (FRIN), Ibadan, were voucher specimens were deposited under FHI 106920.

Extraction of oils

Essential oils were determined by hydrodistillation (4 h) of the air-dried plant materials using a Clevenger-type apparatus in accordance with the *British Pharmacopoeia* (1980) The oils were dried over sodium sulfate and kept in refrigeration (4°C) after estimation of percentage yields.

Analysis of essential oils

The oils extracted were subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector (MSD), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase (30 m $\times$ 0.25 μm film thickness). The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 mL/min. inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, held for 10 min; increased at 3°C/min to 200°C; increased 2°C/min to 200°C. The sample was dissolved in CH_2Cl_2, and a split injection technique was used.

Identification of constituents present in oils

Identification of individual constituents of the essential oils was achieved based on their retention indices (determined with a reference to a homologous series of normal alkanes) and by comparison of their mass spectral fragmentation patterns (NIST database/ChemStation data system) and with literature (Adama, 2001; Martins et al., 2001).

Larval collection

Larvae were collected at Ojoo area, Ibadan, Oyo state, Nigeria, from tire-print breeding sites and reared in plastic bowls containing clean well water. They were fed with dog biscuit.

Larval toxicity assay of essential oils

Stock solutions of each of the oils were prepared at 100 mg/mL with ethanol. Test solutions of oils at 50, 25, 12.5, and 6.25 mg/mL were prepared by serial dilution of the stock solution with ethanol. Sterile disposable cups (250 mL) were used for the study. Test oils (1 mL) were introduced into a cup containing dechlorinated water (99 mL), making the final test concentrations 1, 0.5, 0.25, 0.125, and 0.063 mg/mL, respectively. Twenty fourth-Instar larvae were released into each cup containing 100 mL solution of each test concentration. After 24 h, the number of dead larvae in each cup was counted (assessed by inability of larva to get to the water surface when agitated). Oil was considered toxic if it had 100% mortality. Experiments showed no significant difference in mortality when the experiment was extended to 48 h. Control experiments carried out with DEET and with 1% ethanol were run in parallel. Experiments were done in four replicates.

Statistical analysis

Results were expressed as means $\pm$ SEM of four independent experiments. Larval toxicities were reported as LC_{50} obtained form GraphPad Prism statistical software.

Results

The percentage yields of essential oils in the leaf and rhizome of *C. longa* were 0.67% w/w and 1.33% w/w, respectively. Chemical analysis of the oils afforded the identification of 13 and 16 constituents, respectively, representing 100% and 99.9% of the total essential oil composition (Table 1). In both oils, sesquiterpenoids were the most abundant terpenoids (95.1%, 99.9%) with the oxygenated derivatives being dominant (91.72%, 94.8%) for the leaves

Table 1. Chemical composition of *Curcuma longa* leaf and rhizome essential oils.

RI[a]	Compounds[b,c]	Percentage composition		QI[d]
		Leaf	Rhizome	
977	β-Pinene	0.1	—	95
1023	*p*-Cymene	1.6	—	97
1029	1,8-Cineole	1.6	—	98
1417	β-Caryophyllene	—	0.2	99
1411	*cis*-β-Farnesene	—	0.2	—
1453	α-Humulene	0.2	Tr	97
1459	*trans*-β-Farnesene	—	Tr	90
1483	ar-Curcumene	2.0	1.8	99
1487	β-Lonone	0.6	—	96
1496	α-Zingiberone	—	1.1	99
1509	β-Bisabolene	0.3	0.3	98
1524	β-Sesquiphellandrene	0.9	1.5	98
1579	ar-turmerol	—	1.3	—
1588	Dihydro-ar-turmerone	—	0.2	—
1633	β-Acorenol	1.0	—	—
1670	ar-Turmerone	63.4	44.4	90
1673	β-Turmerone[e]	12.6	26.5	98
1703	α-Turmerone[e]	13.7	20.8	94
1743	6*R*,7*R*-Bisabolone[f]	—	0.4	—
1770	(*E*)-α-Atlantone	1.0	0.4	90
1792	α-Bisabolol acetate	—	0.8	—
Total		100.0	99.9	

–, not detected; Tr, trace <0.1%.
[a]Retention indices on HP-5ms capillary coated column.
[b]Order of elution on HP-5ms capillary coated column.
[c]Identified by comparison of the mass spectrum and retention index data.
[d]QI (Quality Index) reflects the fit comparison of experimental mass spectrum and NIST library mass spectrum.
[e]RI value from Martins et al. (2001).
[f]RI value from Chassagnez-Mendez et al. (2000).

Table 2. Larvicidal activities of the leaf and rhizome oils of *C. longa* on *An. gambiae* larvae.

Concentration (mg/ml)	Larval mortality ± SEM*		
	Leaf	Rhizome	DEET
2.0	—	—	100 ± 0.0
1.0	100 ± 0.00	100 ± 0.00	45.0 ± 1.0
0.50	100 ± 0.00	100 ± 0.00	37.5 ± 2.0
0.25	95.0 ± 1.00	100 ± 0.00	30.0 ± 2.5
0.125	76.5 ± 4.76	100 ± 0.00	20.0 ± 0.5
0.063	63.7 ± 3.27	85.0 ± 2.60	
0.0312	52.5 ± 0.00	85.0 ± 1.00	
0.016	12.5 ± 0.50	32.5 ± 1.53	
0.008	0.00 ± 0.00	2.50 ± 0.50	
1% Ethanol	0.00 ± 0.00		

*Results were expressed as the mean ± SEM of four independent experiments.

and rhizomes, respectively. The oil of the rhizome was the more active of the two oils tested. The oils of the rhizome and leaves produced 100% mortality at 0.125 and 0.500 mg/mL, with LC_{50} values of 0.017 and 0.029 mg/mL respectively, as displayed in Table 2. In this study, the LC_{50} of DEET was 1.09 mg/mL.

Discussion

A comparison of the composition pattern of both oils reveals some quantitative and qualitative variations. Of a total of 21 constituents identified, eight were found in both oils. It has been reported that the quantitative essential oil composition is widely influenced by the genotype, ontogenic development, and environmental and growing conditions (Piccaglia et al., 1991; Shu & Lawrence, 1977). This suggests the possibility of different composition and uses of plant species grown in different regions. The abundance of turmerone in the chemical composition of the essential oils of Nigerian-grown *C. longa* makes it similar to earlier reports for the Indian and Pakistani samples (Gurdip et al., 2002; Riaz et al., 2000). Other workers reported low concentrations of turmerone with high monoterpenoid content in the *C. longa* essential oils in other regions (Banal et al., 2002; Martins et al., 2001; Sakuntala et al., 2002). The fresh leaf essential oil of C. *longa* grown in Ile-Ife, in southwest Nigeria, with predominaetly tropical rainforest vegetation, was reported to contain α-phellandrene (47.7%) and terpinolene (28.9%) as major constituents (Oguntimein et al., 1990). In the current study, the leaf was sourced from South-South zone, in the Cross-River region. These notable variations in chemical composition of the leaf essential oil may be attributed to their geographical locations.

To the best of our knowledge, pipericide, a constituent of *Piper nigrum* fruit has been reported to be the most toxic botanical compound to *Culex pipens pallens* larvae with an LC_{50} of 0.004 mg/L (Park et al., 2002). The lowest and most promising dose for a crude extract reported was 0.69 mg/L recorded for a steam-distilled extract of *Callitris glaucophylla* against *Aedes aegypti* (Shaalan et al., 2003). These low doses are comparable with many synthetic insecticides.

Identification of novel effective mosquitocidal compounds is imperative because of increasing resistance of mosquitoes to currently used insecticides, concern for the environment and food safety, the unacceptability of many organophosphates and organochlorines, and the high cost of synthetic pyrethroids (Shaalan et al., 2005).

Activity displayed was concentration dependent and the results better than the toxicity displayed by DEET, indicating that *C. longa* leaf and rhizome essential oils could serve as alternatives to synthetic larvicides and a means of malaria vector control.

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References

Abbiw DK (1990): *The Useful Plants of Ghana*. Kewi, Royal Botanical Gardens, p. 52, 108.

Adama RP (2001): Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL, Allured Publ., pp. 25–86.

Ansari MA, Razdan RK (1994): Repellent action of *Cymbopogan martini martini* Staf. var *sofia* against mosquitoes. *Ind J Malariol 31*: 95–102.

Ansari MA, Razdan RK (1995): Relative efficacy of various oils in repelling mosquitoes. *Ind J Malariol 32*: 104–111.

Ansari MA, Vasudevan P, Tendon M, Razdan RK (1999): Larvicidal and mosquito repellent action of peppermint (*Mentha piperita)* oil. *Biores Technol 71*: 267–271.

Banal RP, Bahl JR, Garg SN, Naqvi AA, Sushil K, Kumar S (2002): Differential chemical composition of the essential oils of the shoot organs, rhizomes and rhizoids in the turmeric *Curcuma longa* grown in Indo-Gangetic plains. *Pharm Biol 40*: 384–389.

Breman JG, Egan A, Keusch GT (2001): The intolerable burden of malaria: A new look at the numbers. *Am J Trop Med Hyg 64*(1–2 Suppl): 1–11.

British Pharmacopoeia (1980): Vol. II, London, H.M. Stationery Office, London, Pharmaceutical Press, p. 109.

Chane-Ming J, Vera R, Chalchat JC, Cabassu P (2002): Chemical composition of essential oils from rhizomes, leaves and flowers of *Curcuma longa* L. from Reunion Island. *J Essent Oil Res 14*: 249–251.

Chassagnez-Mendez AL, Machado NT, Araujo ME, Maia JG, Meireles MA (2000): Supercritical CO_2 extraction of curcumins and essential oil from the rhizomes of turmeric (*Curcuma longa* L.). *Ind Eng Chem Res 39*: 4729–4733.

Fradin MS, Day KF (2002): Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med 347*: 13–17.

Gurdip S, Om PS, Sumitra M (2002): Chemical and biocidal investigation on essential oil of some Indian *Curcuma* species. *Prog. Crystal Growth Characteriz Mater 45*: 75–81.

Martins AP, Slgueiro L, Gonclaves MJ, Cunha AP, Vila R, Canogueral S, Mazzoni V, Tomi F, Casanova J, Da-cunha AP (2001): Essential oil composition and antimicrobial activity of three Zingiberaceae from Sao Tome and Principe. *Planta Med 67*: 580–584.

Oguntimein BO, Weyerstahl P, Marschali-Weyrstahl H (1990): Essential oil of *Curcuma longa* L. leaves. *Flav Frag J 5*: 89–90.

Pandian RS, Kathiresan K (2002): Repellent activity of the rhizome of *Curcuma longa* Linn. against nocturnal mosquitoes in a rural area. *Insect Environ 8*: 127–129.

Park I, Lee S, Shin S, Park J, Ahn Y (2002): Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. *J Agric Food Chem 50*: 1866–1870.

Piccaglia R, Marotti M, Galletti GC (1991): Characterization of essential oil from a *Satureja montara* L. a chemotype grown in northern Italy. *J Essen Oil Res 3*: 147–152.

Rambir S, Ramesh C, Mridula B, Luthra PM, Singh R, Chandra R, Bose M (2002): Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. *Curr Sci 83*: 737–740.

Riaz M, Iqbal MJ, Chaudhary FM (2000): Chemical composition of the volatile oils from rhizomes of *Curcuma longa* Linn. of Pakistan. *Bangladesh J Sci Indus Res 35*: 163–166.

Sacchetti G, Maietti S, Muzzdi M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005): Comparative evaluation of 11 essential oils of different origins as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem 91*: 621–632.

Sakuntala B, Sahoo S, Srivastava VK, Behura S (2002): Major constituents in leaf essential oils of *Curcuma longa* L. and *Curcuma aromatica* Salisd. *Curr Sci 83*: 1312–1313.

Shaalan E, Canyon DV, Faried MW, Abdel-Wahab H, Mansour A (2003): Efficacy of a highly active phytochemical (*Callitris glaucophylla*) against mosquito vectors of dengue and Japanese encephalitis. The Annual Queensland Health and Medical Scientific Meeting, "Making It Better: Encouraging Health Research and Innovation," 25– 26 November, Brisbane.

Shaalan EA, Canyon DV, Faried MW, Abdel-Wahab H, Mansour A (2005): A review of botanical phytochemicals with mosquitocidal potential. *Environ Intern 31*: 1149–1166.

Shu CK, Lawrence BM (1977): Reason for the variation in composition of some commercial essential oils. In: Risch SJ, Ho CT, eds. *Spices, Flavour Chemistry and Antioxidant Properties*. ACS Symposium Series, *American Chemical Society,* Vol. 660. Washington, DC, p. 138.

Tawatsin A, Thavara U, Techadamrongsin Y, Chompoosri J, Kongngamsuk W (2001): Ovipositional deterrence, larvicidal and repellency effects of turmeric (*Curcuma longa* Linn.) and galangle minor (*Alpinia officinrum* Hance) against four mosquito vectors. 3rd International Congress of Vector Ecology, Barcelona, Spain.

Thorne-Research (2001): Curcuma longa (Tumeric) Monograph. *Alternative Medicine Review 6*: 26–66.

Tripithi AK, Prajapati V, Verma N, Bahl JR, Banal PR, Khanuja SPS, Kumar S (2002): Bioactivities of the leaf essential oil of *Curcuma longa* (Vare. Ch-66) on three species of stored-product beetles (Coleopatra). *J Econ Entomol 95*: 183–189.

WHO World Health Report (1998): *Life in the 21st Century: A vision for all*. No. 90–104. Geneva, WHO.

WHO Expert Committee on Malaria (2000): World Health Organization Technical Report Series. *World Organ Tech Rep Ser 892*: I-V, 1–74.