

Research Article

Repellent Activity of Bark Extracts of *Zanthoxylum heitzii* Against the Malaria Vector *Anopheles gambiae*

Bertin Mikolo^{1*}, Hans J Overgaard², Daniel Massamba¹, Fabrice Chandre³, Marie Rossignol³, Stephane Duchon³, Karl E Malterud⁴, Berit S Paulsen⁴ and Vincent Corbel⁵

¹Ecole Nationale Supérieure Polytechnique, Université Marien Ngouabi, BP 69 Brazzaville, République du Congo

²Department of Mathematical Sciences and Technology (IMT), Norwegian University of Life Sciences (NMUB), Ås, Norway

³Laboratoire de lutte contre les Insectes Nuisibles (LIN), IRD, Montpellier, France

⁴Department of Pharmacy, Section Pharmaceutical chemistry, Area Pharmacognosy, University of Oslo, Norway

⁵Laboratory of insecticidal bioassays, IRD-CREC, Cotonou, Benin

Abstract

The objective of this study was to evaluate crude extracts of leaves, bark and seeds of the plant *Zanthoxylum heitzii* using hexane, ethyl acetate, ethanol, water/ethanol and water solvents, and to evaluate the extracts for repellence to the malaria mosquito, *Anopheles gambiae*.

The results obtained showed that extracts from the bark of the native plant *Zanthoxylum heitzii*, unlike that of seeds and leaves, contain repellent compounds against *Anopheles gambiae*. The most active extract was obtained with hexane from the bark, while other solvents gave less active extracts. The Kdr strain of *Anopheles gambiae* mosquitoes was the most sensitive, followed by Kisumu and with Acer as the least sensitive strain. Effects of leaf and seed extracts with either hexane or other solvents were not significant.

Keywords: Mosquitoes; Repellence; Solvents; *Zanthoxylum*

*Corresponding author: Bertin Mikolo, Ecole Nationale Supérieure Polytechnique, Université Marien Ngouabi, BP 69 Brazzaville, République du Congo, Tel: +242 068641803; E-mail: mikolobertin@yahoo.fr

Citation: Mikolo B, Overgaard HJ, Massamba D, Chandre F, Rossignol M, et al. (2019) Repellent Activity of Bark Extracts of *Zanthoxylum heitzii* Against the Malaria Vector *Anopheles gambiae*. J Plant Sci Curr Res 3: 009.

Received: October 26, 2019; **Accepted:** December 12, 2019; **Published:** December 19, 2019

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Introduction

Control of insect vectors of diseases remains one of the great concerns in public health, particularly in tropical countries. Until now, insecticidal compound application is one of the main measures recommended by WHO for this purpose. However, due to the side effects of insecticide overuse causing resistance in mosquitoes, intoxication to humans, and environmental damage, efforts are made to produce safer and cheaper chemicals including repellents in addition to insecticides. Many insecticidal and repellent plant materials and some pathogens have been tested on insect vectors of diseases [1]. The purpose is to avoid insect bites either by killing or by repelling the vectors. Bites are currently avoided with insect repellents; most of them made with DEET (N,N-diethyl-m-toluamide) or botanical oils [2]. Different methods used for mosquito repellent applications are reported by Patel et al. [3].

Among the plants studied for their repellent or insecticidal properties are those belonging to the *Zanthoxylum* genus (also named *Fagara*) of the Rutaceae family [4,5]. *Zanthoxylum* is mainly found in tropical regions around the world and is a rich genus with up to 250 species identified. Many of these species are sources of materials used for medicine or pest management purposes. Bioactive constituents of *Zanthoxylum* genus have been reviewed [6].

In Central Africa, a well known species is *Z. heitzii* (Aubrev. & Pellegr.) P.G. Waterman; syn. *Fagara heitzii* Aubrev. & Pellegr. [7] (Figure 1) and so is *Zanthoxylum gillettii* (De Wild) P.G. Waterman (syn. *Fagara macrophylla* (Oliv.) Engl.), also found elsewhere. The bark of *Z. heitzii* (Figure 2) is toxic to the American cockroach (*Periplaneta americana*), stored product pests [8] and mosquitoes [9]. A review of *Zanthoxylum* species used for medicinal purposes in East Africa can be found in Lye et al. [10]. Other species of this genus such as *Z. amratum* and *Z. alatum* can be found in Asia and the Americas.



Figure 1: *Zanthoxylum heitzii* (By: Ehoarn Bidault, CC BY-NC-ND 4.0).

These plants are traditionally used for controlling a wide range of pests and diseases including mosquitoes, beetles, malaria, sickle-cell anemia and toothache. The plant parts used most often are the bark of stems and roots [11-13].

Because of their traditional use, the plants of this genus have been subject to laboratory investigations. Dried plant materials (leaves,

bark and fruit powder), crude extracts and their fractions, isolated compounds, and their synthetic analogues have been tested on insects. These plant materials have been investigated either alone or in mixtures to study potential synergism or antagonism. Extracts of different *Zanthoxylum* species are used in mixture with other plant materials in pesticide preparations. Most of them have been performed in China. Those prepared with extracts from *Z. piperitum* [14], *Z. bungeanum* [15-18] and *Z. usambarensense* [19] have expressed biological activities on some insect pests and microorganisms.



Figure 2: Bark of *Zanthoxylum heitzii* (By K.E. Malterud, reproduced by permission).

The objective of this study was to test *Z. heitzii* crude leaf, bark and seed extracts for repellence against *A. gambiae* and to determine which of hexane, ethyl acetate, ethanol and water were the best solvent for extraction of repellent compounds.

Materials and Methods

Botanical material preparation

Leaves, seeds, and bark of *Z. heitzii* were collected around Douakani village in the southwestern part of the Republic of Congo. The plant material was treated and extracts were made as previously described [9]. Briefly, after drying, grinding and extraction with hexane in a Soxhlet apparatus or in an Accelerated Solvent Extractor (ASE) with hexane (Soxhlet, ASE) followed by ethyl acetate, 96% ethanol, 50% water/ethanol, and water (ASE), extracts were filtered, taken to dryness *in vacuo* or freeze-dried.

Mosquito strains

Three strains of *Anopheles gambiae* s.s. Giles were used in this study:

1. **Kis**: Kisumu strain originating from Kenya is free of any detectable insecticide resistance mechanisms.
2. **kdrKis**: A pyrethroid and DDT resistant strain, obtained by introgression of L1014F (kdr mutation) into the genome of the susceptible Kisumu strain through successive backcrosses followed by selection with permethrin (1 mg/L). kdrKis has the same genetic background as the Kisumu strain, but differs by the presence of 1014F allele at homozygous state.
3. **AcerKis**: An organophosphate and carbamate resistant strain, obtained by introgression of insensitive acetylcholinesterase (Ace1R) into the genome of the Kisumu strain through successive backcrosses followed by selection with propoxur (10 mg/L). **AcerKis** has the same genetic background as the Kisumu strain, but differs by the presence of Ace1R allele (9S) at homozygous state.

Mosquitoes were kept in the Institut de Recherche de Developpement (IRD), Montpellier, France as previously described [9].

Concentration preparation and paper impregnation

Concentrations of 0% (control), 0.5%, 1%, and 2% were prepared according to the WHO procedures [20]. Concentrations are expressed as percentage of the compound mass per silicone oil mass. An appropriate amount of each extract (13.2 mg extract for 2% concentration, 6.6 mg for 1%, and 3.3 mg for 0.5%) was first diluted in 1.34 ml of acetone, and then mixed with 0.66 ml (648 mg) of silicone oil. The solutions were applied to Whatman filter paper. The impregnated paper was then air-dried for 24 hours and inserted into a cylinder for repellency tests.

Contact repellence tests

Tests were done in LIN (Laboratoire de Lutte contre les Insectes Nuisibles), IRD, Montpellier, following a procedure described in Kawada et al. 2014 [20]. Briefly, tests were carried out in standard WHO susceptibility test kits with exposure tubes lined with Whatman papers impregnated with extract preparations or with solvent alone. Ten mosquitoes were introduced into an observation tube lined with unimpregnated filter paper [21]. The observation tube was attached to an exposure tube, but with a door preventing mosquito movement between tubes. Tubes were placed horizontally on a bench in a quiet room at 22°C and left for 2 min for mosquito stabilization. In these conditions, mosquitoes normally stayed on the inner surface of the observation tube if they were not disturbed. Then doors between observation and exposure tubes were opened carefully to allow mosquitoes free movement between tubes. After 10 min of mosquito contact with the impregnated paper, the door between tubes were closed carefully and numbers of mosquitoes in each tube counted. Each assay was replicated three times.

Data analysis

Concentrations were converted into logarithms. Probit was chosen as a mathematical model. The goodness-of-fit was tested by Chi-square tests. The relative repellence rates expressed by effective concentrations of extracts at 50% response level (EC_{50}) were compared by examining their 95% confidence intervals. If the intervals overlap, the lethal doses do not differ significantly. Data were analysed using the PoloPlus program [22]. Graphs of main effects were drawn using Minitab 17.3.

Results

Analysis of the results showed that repellence rates were concentration-dependent (Table 1(A-C); Figure 3).

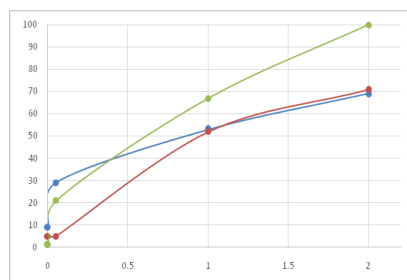


Figure 3: Percent repellence of hexane extract (Soxhlet) of *Z. heitzii* bark as a function of % concentration. Green: kdr-Kis strain (pyrethroid resistant), orange: acer-Kis (organophosphate/carbamate resistant), blue: Kis strain.

Strain	Dose (%)	Soxhlet Hexane		ASE Hexane		ASE Ethyl acetate		ASE Ethanol		ASE Aq.ethanol 50%		ASE Water	
		Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded
Kis	0.00	57	5	62	1	59	3	63	0	60	1	60	1
	0.05	52	15	61	1	71	11	63	9	59	1	63	7
	1.00	60	32	62	4	60	21	66	8	59	1	62	5
	2.00	51	35	65	32	63	15	64	9	60	1	61	7
Acer	0.00	60	3	30	1	30	0	31	0	30	0	30	0
	0.05	57	3	78	16	60	1	62	1	57	0	58	0
	1.00	69	36	60	2	60	1	61	1	59	1	62	2
	2.00	63	45	59	7	66	16	58	5	70	4	61	2
Kdr	0.00	103	1	64	3	18	0	30	2	30	2	31	2
	0.05	68	14	60	3	24	1	31	12	30	3	19	4
	1.00	67	45	62	21	28	7	31	10	19	3	17	6
	2.00	64	64	62	45	21	8	22	13	21	3	21	7

Table 1A: Cumulative numbers of *Anopheles gambiae* repelled by *Zanthoxylum heitzii* Bark extracts.

Strain	Dose (%)	Soxhlet		ASE Hexane		ASE Ethyl acetate		ASE Ethanol		ASE Aq.ethanol 50%		ASE Water	
		Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded
Kis	0.00	28	3	69	4	59	3	63	0	60	1	60	1
	0.05	63	13	58	7	66	5	58	10	59	1	60	1
	1.00	58	16	67	5	63	9	66	7	59	1	59	3
	2.00	62	17	50	10	60	8	65	9	60	1	61	4
Acer	0.00	30	0	30	0	30	0	31	0	30	1	30	0
	0.05	60	2	61	1	66	1	58	0	62	2	62	0
	1.00	60	7	60	2	60	0	60	1	62	2	60	1
	2.00	78	12	64	2	62	0	60	0	62	4	60	0
Kdr	0.00	39	1	32	0	31	2	30	2	28	0	30	2
	0.05	32	2	32	2	30	4	37	7	29	0	22	2
	1.00	30	4	30	2	32	8	32	13	31	3	14	3
	2.00	35	5	32	2	30	8	27	13	33	3	19	2

Table 1B: Cumulative numbers of *Anopheles gambiae* repelled by *Zanthoxylum heitzii* Seed extracts.

Strain	Dose (%)	Soxhlet Hexane		ASE Hexane		ASE Ethyl acetate		ASE Ethanol		ASE Aq. ethanol 50%		ASE Water	
		Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded	Tested	Responded
Kis	0.00	65	2	63	2	63	3	53	2	60	2	60	2
	0.05	60	1	67	3	63	3	44	2	60	2	63	2
	1.00	58	2	69	9	64	4	57	5	62	3	64	3
	2.00	63	3	63	3	64	5	64	2	59	3	59	3
Acer	0.00	60	1	33	1	33	1	31	1	33	1	33	1
	0.05	57	3	62	2	33	1	34	1	30	0	33	1
	1.00	62	5	59	1	29	0	30	0	32	0	33	2
	2.00	61	5	60	0	31	1	34	1	35	1	31	1
Kdr	0.00	58	2	18	0	31	2	28	0	28	0	30	1
	0.05	60	2	26	2	14	5	30	1	31	1	20	2
	1.00	60	3	28	7	18	5	32	2	29	1	24	2
	2.00	50	0	21	8	24	9	28	1	38	5	18	3

Table 1C: Cumulative numbers of *Anopheles gambiae* repelled by *Zanthoxylum heitzii* Leaves extracts.

The hexane (Soxhlet) bark extract showed the highest activity, with more than 50% repellence towards all strains at 1% concentration. All three mosquito strains were sensitive to this extract, according to the chi-square values obtained. The pyrethroid-resistant strain KdrKis was the most sensitive one, followed by Kisumu and AcerKis. The EC₅₀ values obtained with this extract tested on these strains, KdrKis, Kisumu and AcerKis, were 0.28%, 0.84% and 1.03% respectively (Table 2).

Extraction methods	Parameters	Kisumu	Kdr-Kis	Acer-Kis
	LC ₅₀ %m/m silicone	0.841	0.279	1.027
	95%CI	0.533-1.432	0.078-to 0.626	0.557 -1.365
	X ²	10.358	13.857	0.035
	Degree of freedom	9	5	1
	Heterogeneity	1.1509	2.7715	0.035
	EC ₅₀ %V/V	2.035	1.390	50.552
	95%CI	1.805 -2.461	1.152 -1.652	8.653- 695165.502
	X ²	0.000	0.013	0.428
	Degree of freedom	1	1	1
	Heterogeneity	0.000	0.013	0.428

CI = Confidence interval, EC = Effective concentration

Table 2: Analysis of *Z. heitzii* bark extracts repellency to three of *A. gambiae* strains.

All mosquitoes were repelled at a 2% concentration of the hexane (ASE) bark extract, except for the AcerKis strain (12% repellence). The hexane bark extracts obtained by ASE method were less effective towards the KdrKis and Kisumu strains, with EC₅₀ values of 1.4% and 2.0%, respectively.

No significant correlation was present between the extract concentrations and repellence rates for bark extracts obtained with other solvents (ethyl acetate, ethanol and water) and leaf and seed extracts obtained with all solvents.

Discussion

The bark of *Zanthoxylum heitzii* contains compounds which are repellent to three strains of *A. gambiae*. The repellence rates obtained with bark hexane extract were concentration-dependent. The susceptible strain (Kisumu) and the resistant ones (Acer and Kdr) are all susceptible to these extracts. Repellent activity has previously been observed in extracts of related plants such as *Z. zanthoxyloides* on *M. domestica* [23] and in *Z. limonella* (in admixture with clove oil) on *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* mosquitoes [24].

Hexane is the best solvent for extracting *Zanthoxylum heitzii* repellent compounds, thus the active principles are lipophilic, as previously found for the mosquito toxicity of *Z. heitzii* extracts [9]. Egunyomi et al. [25], working on Nigerian plants, also reported that hexane plant extracts were more repellent than methanol ones.

Compounds from seeds, leaves, barks and roots of several *Zanthoxylum* species have been studied for toxicity or repellency towards mosquitoes and other insects. Products often studied are powdered,

dried material, extracts obtained with diverse solvents (water, ethanol, methanol, acetone or hexane) or essential oils. Thus, the essential oil from *Z. setulosum* and the lipophilic extracts from *Z. tetraspermum* and *Z. caudatum* are toxic to aphids [26,30], and *Z. limonella* oil is repellent to mosquitoes [24,27]. Lipophilic extracts of *Z. usambarensis* [19] and *Z. zanthoxyloides* [23] are toxic to the housefly, *Musca domestica*. The essential oils from *Z. limonella* [28], *Z. armatum* [29] and *Z. gillettii* [34] are toxic to mosquito larvae (*Anopheles*, *Aedes* and *Culex*). Larvicidal effects towards mosquitoes is exhibited by the ethanol extract of an unidentified *Zanthoxylum* species [12], as well.

The chemistry of *Z. heitzii* has been investigated, and alkaloids [35-39], amides [37,39,40], phenylethanoids [37], lignans [37-40], triterpenoids [38,39] and sesquiterpenoids [39,40] have been reported. Other *Zanthoxylum* (*Fagara*) species have been investigated, as well (review, e.g. [6]). Many of the compounds reported exhibit medicinal and pesticidal activities such as insecticidal, larvicidal, molluscicidal, fungicidal and antiplasmodial.

The results reported in this communication represent a continuation of our work on antimalarial properties of *Z. heitzii* extracts and constituents [9,39-41]. We conclude that this plant shows promising activity, having antimosquito, antiplasmodial and mosquito repellent properties, and it acts on both chloroquine-sensitive and chloroquine resistant strains of *A. gambiae*. Further work to elucidate its possibility as a drug in practice is needed.

Conclusion

Results of this study showed that the lipophilic extracts from *Z. heitzii* bark obtained with hexane was the most effective repellent material compared to the other extracts obtained using ethyl acetate, ethanol and water as extraction solvents. There was no significant contact repellence observed with the seed and leaf extracts. Among the three strains tested, the resistant strains called Kdr Kis was the most sensible unlike the two other strains Kisumu and AcerKis.

Acknowledgments

Research Council of Norway (RCN) for funding the project. Jasmin MBA| NI from Douakani village for botanical material collecting.

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