

# Adulticide activity in fractions of *Zanthoxylum heitzii* bark extract against *Anopheles gambiae* s.s.



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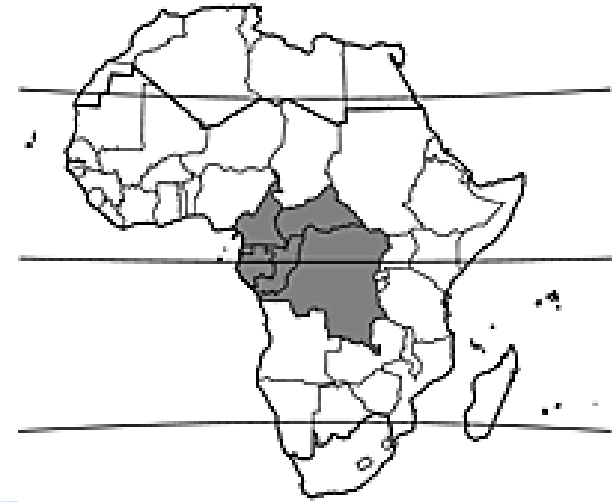
**6<sup>th</sup> European Mosquito Control Association Workshop, Budapest, Hungary 12-15 September 2011**

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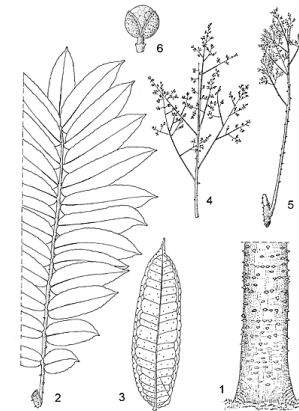
# The Congo Rainforest

- 2<sup>nd</sup> largest tropical rain forest in the world
- A source of potentially useful plants species
- Bioprospecting and research needed
- Ecology and geographical distribution
- Sustainable harvesting



# The Olon tree – *Zanthoxylum heitzii*

- Rutaceae (Citrus family)
- Traditionally used for killing fish
- Flea control on human feet
- Bark extract toxic to prostate cancer cells



- Hexane extracts of *Z. heitzii* bark has insecticidal properties<sup>1</sup>

– Cockroaches, Bruchids (Cowpea weevil, Maize weevil), houseflies

- Preliminary results indicate *Z. heitzii* bark also kills malaria mosquitoes



Photo: P. Pollecot, CIRAD

<sup>1</sup>Mikolo et al. 2009. Extracts from the bark of *Fagara heitzii* (Aubr. et Pel.) (Rutaceae) tree are toxic to two weevils and the American cockroach. *Entomological Research*, 39: 401–405.

# Objectives

- Determine chemical fractions with insecticidal activity in bark extract of *Zanthoxylum heitzii* against *Anopheles gambiae* adults
- Identify compounds in those fractions

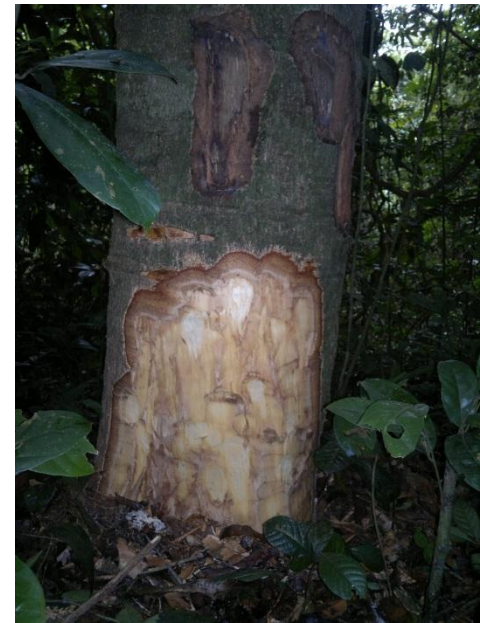


# Methods – Field

1. Bark collections
2. Dried at room temperature for 7 d.
3. Ground to powder (4mm sieve)



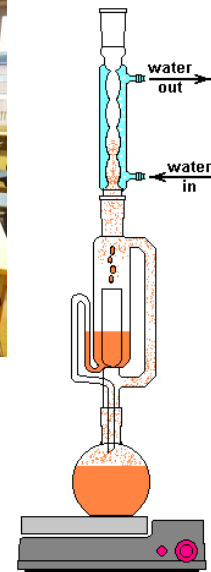
Massif du Chaillu,  
Republic of Congo



# Methods – Laboratory

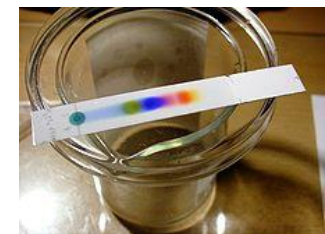
## 1. Extraction

- Soxhlet extraction with hexane
- Extract filtered, concentrated in a rotary evaporator, and vacuum dried (300 g of bark → 3.84 g of crude extract)



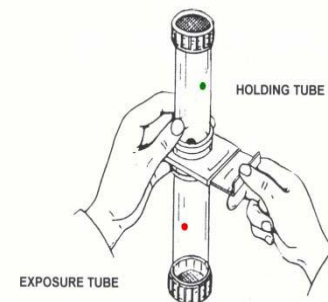
## 2. Fractionation

- Versapak Column Chromatography (Si-gel); elution in an increasing polar gradient
- Analytical Thin Layer Chromatography (TLC)
- Visualized by UV light and/or cerium sulfate spray + heating



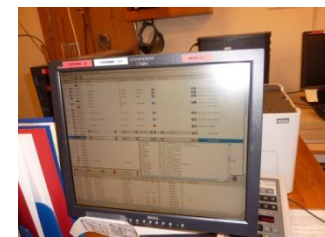
## 3. Insecticide bioassays

- WHO susceptibility bioassays on *A. gambiae* s.s.
- LC<sub>50</sub> calculated by probit analysis using PoloPlus software

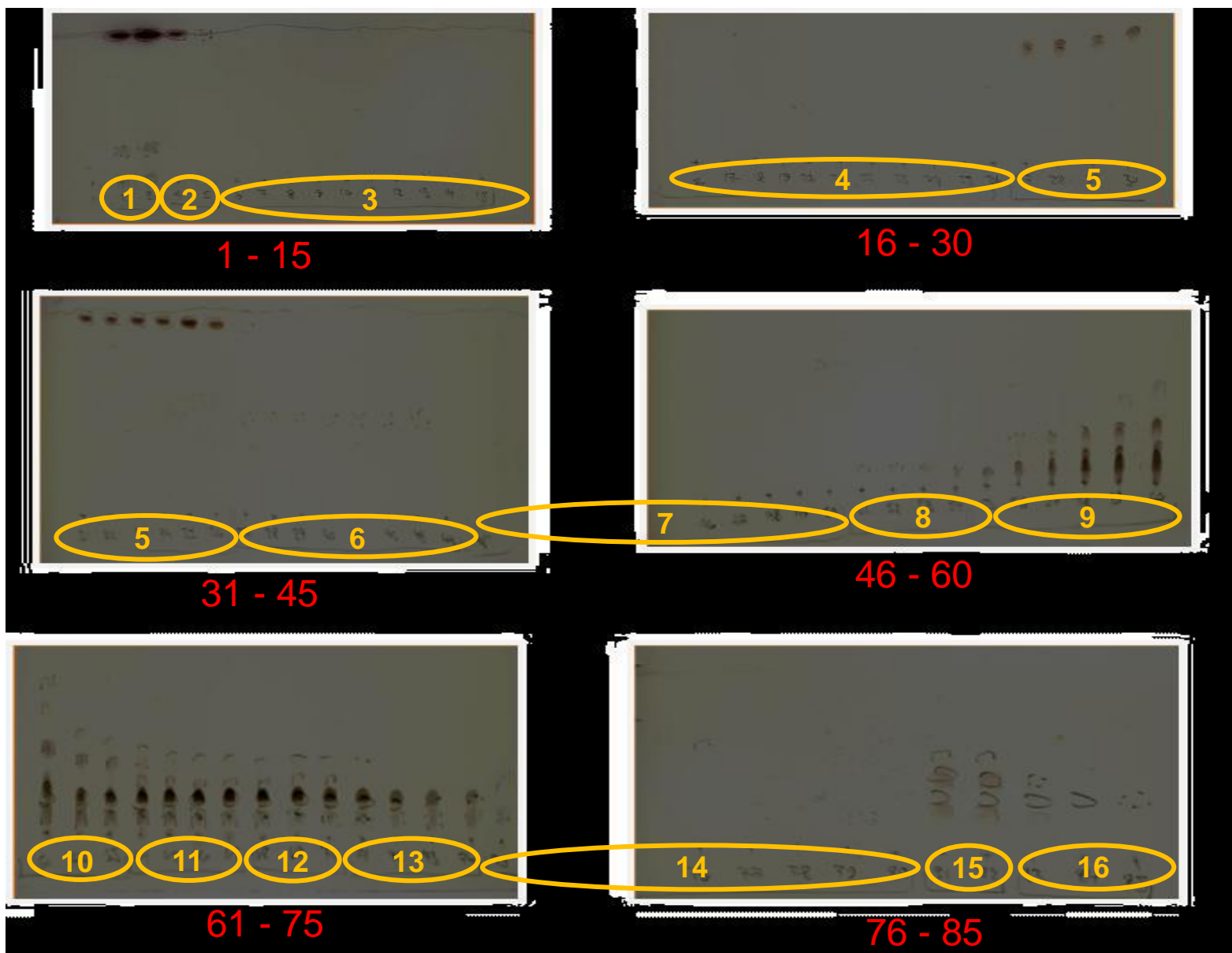


## 4. Identification of compounds

- Nuclear magnetic resonance (NMR) for structure elucidation

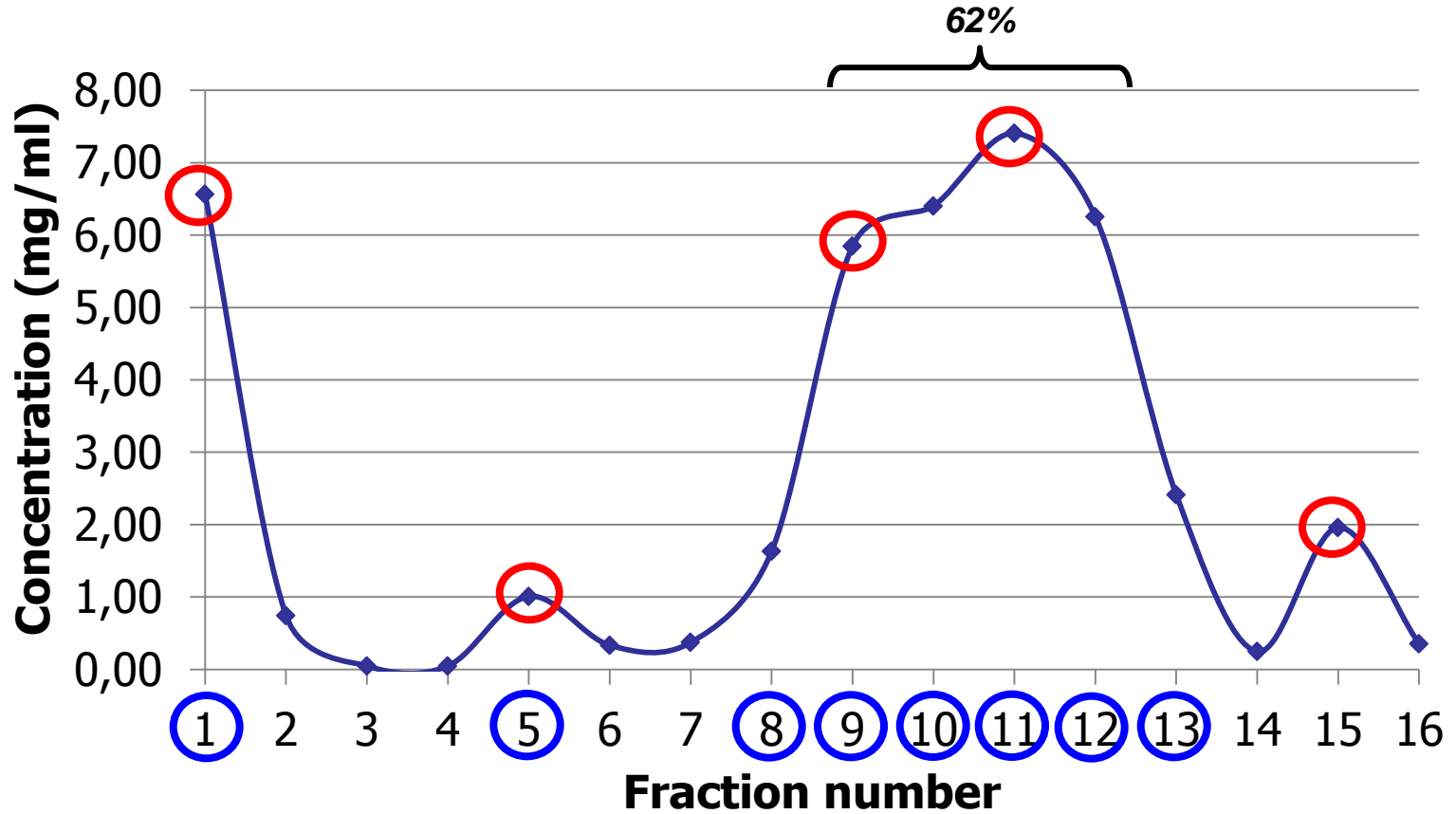


# Fractionation of crude extract





# Fraction concentrations in crude extract



- Concentration peaks
- Tested on *An. gambiae* s.s.



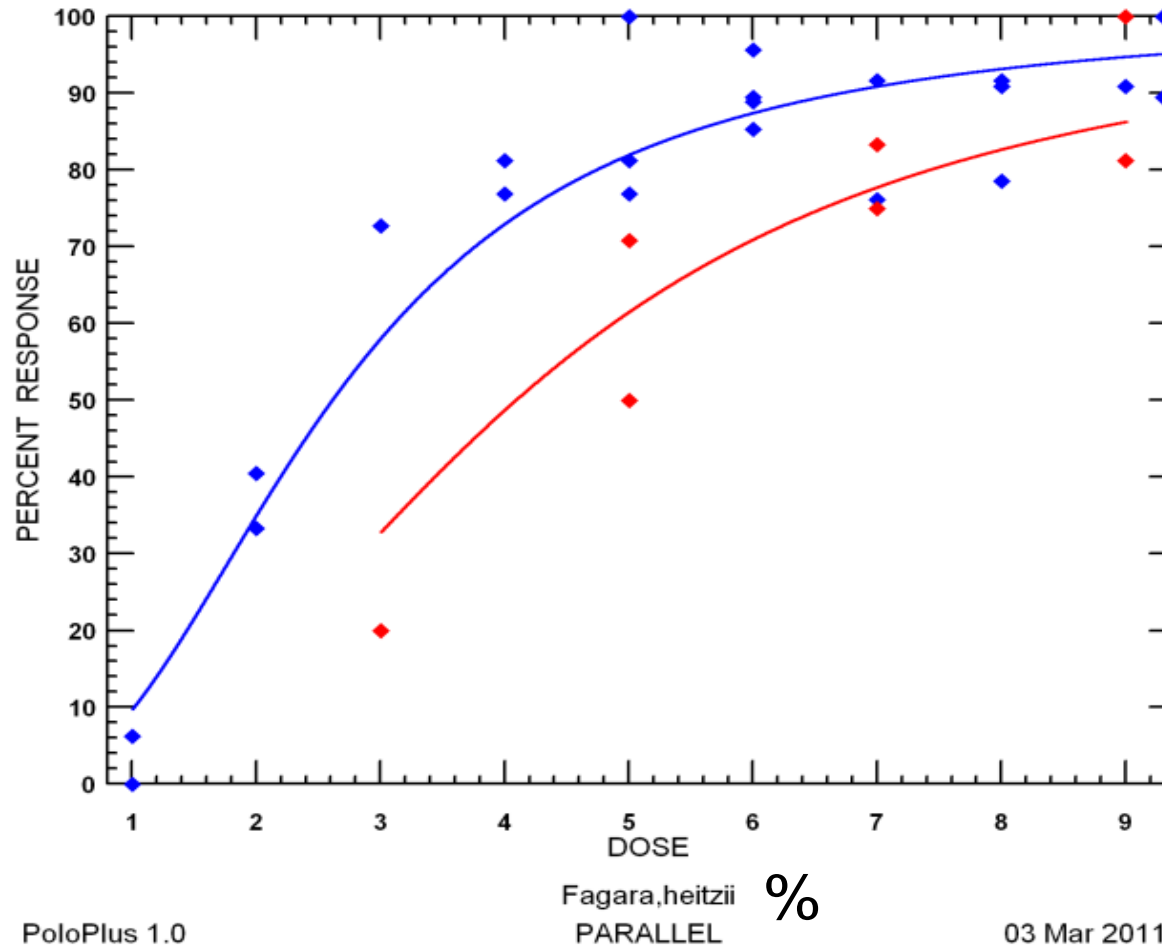
# Bioassays

Crude extract

Fraction 11 (F11)



# Mortality of *A. gambiae* s.s.



Crude extract



Fraction 11  
(F11)



$\chi^2=19.91$   
df=2,  
p<0.05

PoloPlus 1.0

Fagara, heitzii %  
PARALLEL

03 Mar 2011

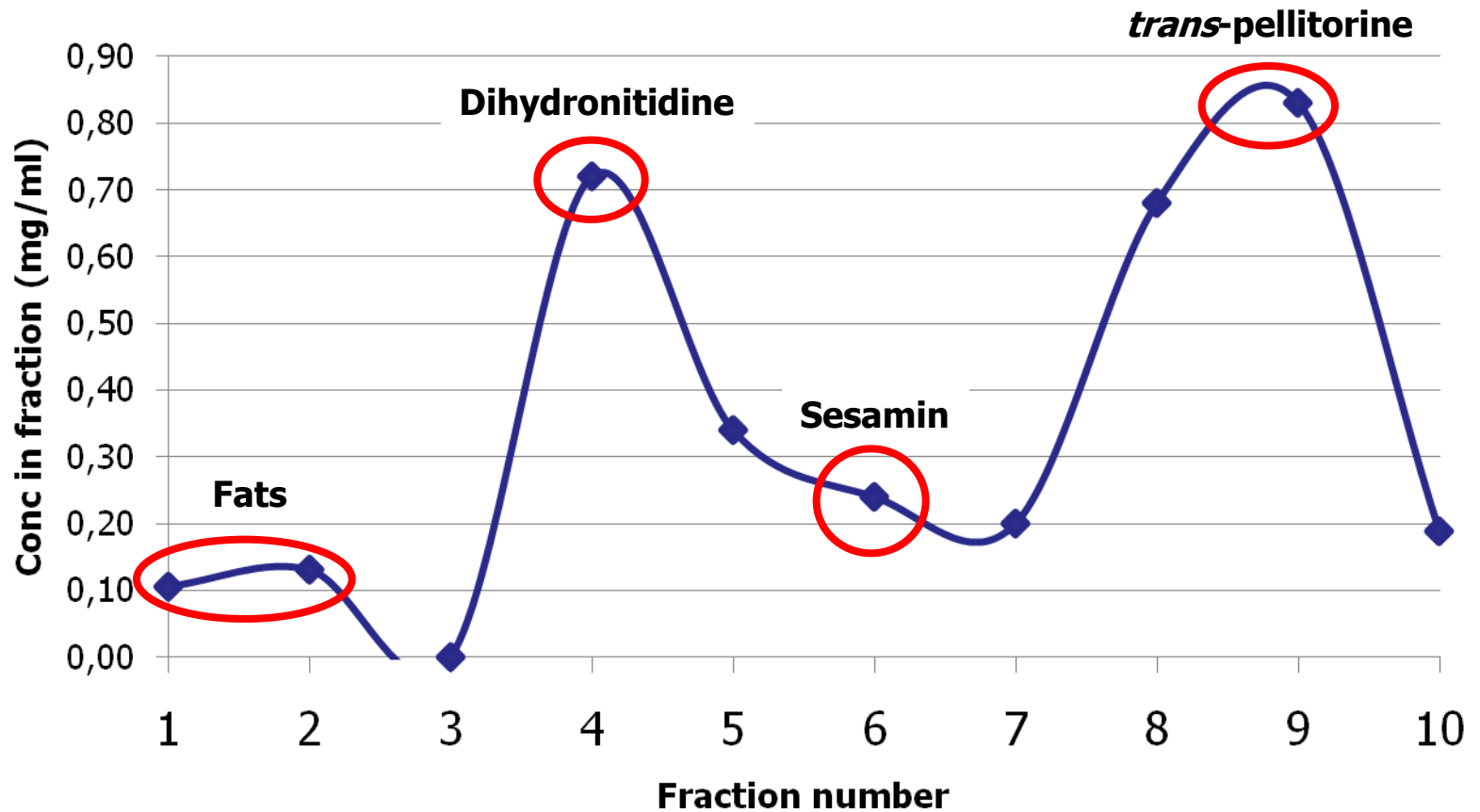


# Lethal concentrations

Compound	df	$\chi^2$	LC <sub>50</sub> (%)	LC <sub>50</sub> 95% C.I.	
				Lower	Upper
Crude extract	23	31.21	2.53	2.04	3.01
Fraction 11	5	6.93	4.47	3.02	5.39

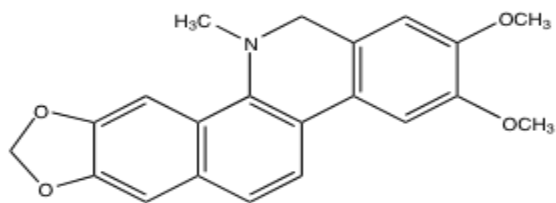
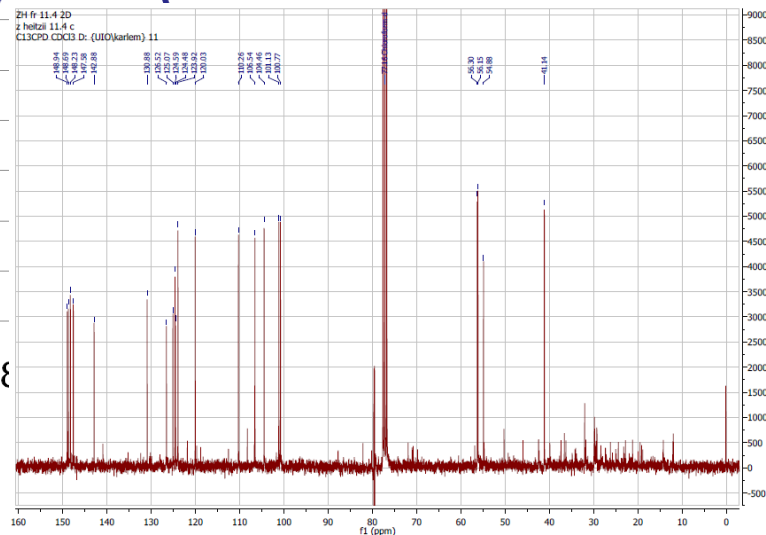
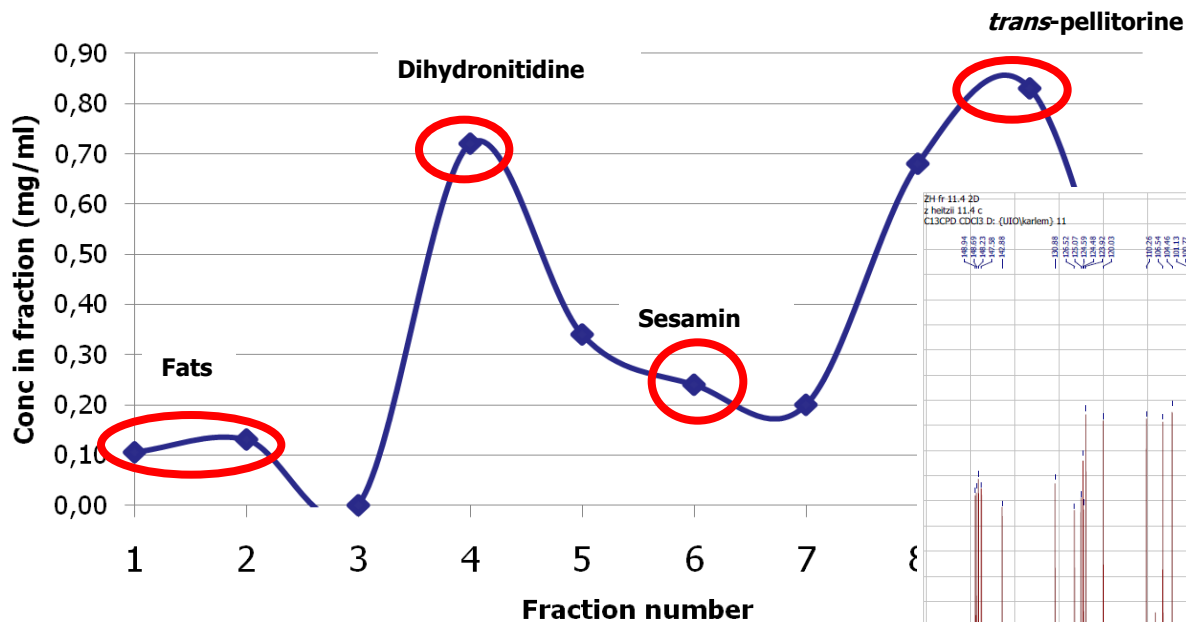
# Fraction concentrations in F11

- 50 fractions combined into 10 fractions
- 4 major peaks

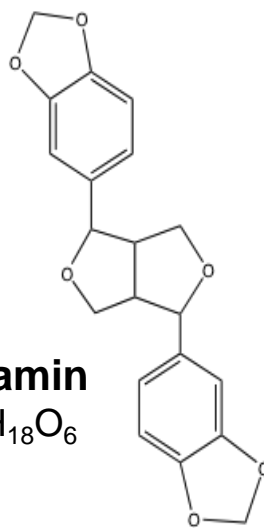




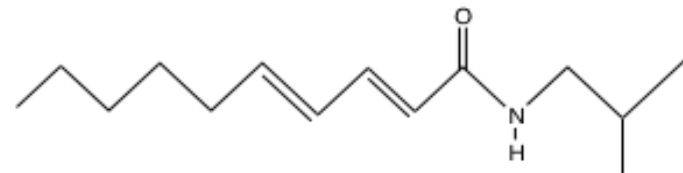
# Structure elucidation of identified compounds



**Dihydronitidine**  
C<sub>21</sub>H<sub>19</sub>NO<sub>4</sub>



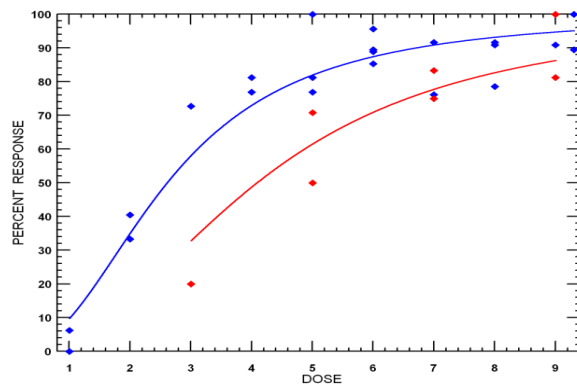
**Sesamin**  
C<sub>20</sub>H<sub>18</sub>O<sub>6</sub>



***trans*-pellitorine**  
C<sub>14</sub>H<sub>25</sub>NO

# Conclusions 1

- Insecticidal activity in *Z. heitzii* against *A. gambiae* adults confirmed
- Mortality regression lines of crude extract and F11 parallel
  - Major toxic ingredient likely the same
- Crude extract > F11
  - Synergistic/additive activity with other molecules in other fractions
  - Interference due to repellent products (*surviving mosquitoes on top of tube net*)



PoloPlus 1.0

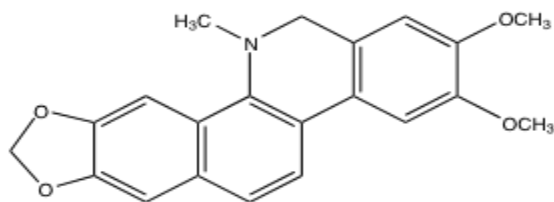
Fagara.heitzii  
PARALLEL

03 Mar 2011

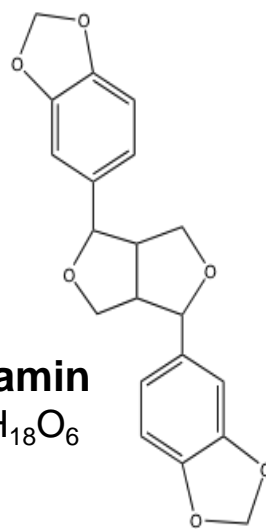


## Conclusions 2

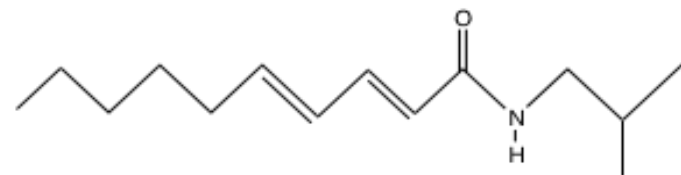
- First time **dihydronitidine** and **trans-pellitorine** have been described in this plant
- **Dihydronitidine**: has antiplasmodial, antileishmanial and anti-cancer activity, but first time reported as insecticidal
- **Sesamin** and **trans-pellitorine** has insecticidal activities



**Dihydronitidine**  
 $C_{21}H_{19}NO_4$



**Sesamin**  
 $C_{20}H_{18}O_6$



**trans-pellitorine**  
 $C_{14}H_{25}NO$

## Further studies needed

- Additional extraction and fractionation to obtain more material
- Topical bioassays
- More rigorous WHO bioassays
- Repellency assays
- Synergism
- Geographical distribution and sustainable harvesting

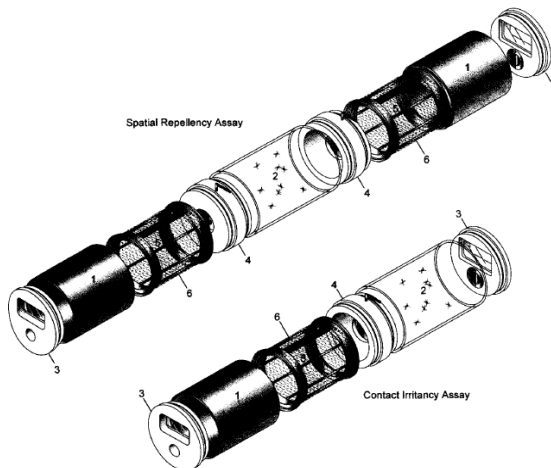


Photo: Abrahan Matias

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# Thank you for your attention!

