ANTI-PLASMODIAL QUINONES FROM THE RHIZOMES OF *KNIPHOFIA FOLIOSA*

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Malaria

- An endemic and deadly disease (Casteel, 2003)
- Been noted for more than 4000 years
- Vector: The female *Anopheles* mosquito (Pillay *et al.*, 2008)
Malaria: statistics

- Distribution: distributed in 101 countries, 45 in sub-Saharan Africa (Casteel, 2003)
- Between 300-500 million cases of malaria infections every year (WHO, 2009)
- 1.5 – 2.7 million deaths every year (Casteel, 2003)
- In Africa south of Sahara, 75% of deaths are children under five (Africa Malaria Report, 2003)
Malaria: More information

- Major public health and economic problem in tropical and sub-tropical region (Kaur et al. 2009)
- Besides causing up to 2 million deaths, causes unimaginable suffering and disability and leads to economic loss (Sachs and Malanay, 2002)
Prevalence of malaria

Taylor et al. 2007
PROBLEM STATEMENT
## Resistance

<table>
<thead>
<tr>
<th>Anti-malarial drugs</th>
<th>Plasmodium strain</th>
<th>Resistance first reported (year)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td><em>P. falciparum</em></td>
<td>1910</td>
<td>ICMR bulletin, 2008; Gregson and Plowe, 2005; Bloland, 2001;</td>
</tr>
<tr>
<td>Chloroquine</td>
<td><em>P. falciparum</em></td>
<td>1960</td>
<td>ICMR bulletin, 2008; Bloland, 2001</td>
</tr>
<tr>
<td></td>
<td><em>P. vivax</em></td>
<td>1989</td>
<td>ICMR bulletin, 2008; Mendis <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Sulphadoxine/pyrimethamine</td>
<td><em>P. falciparum</em></td>
<td>1967</td>
<td>ICMR bulletin, 2008; Bloland, 2001</td>
</tr>
<tr>
<td>Mefloquine</td>
<td><em>P. falciparum</em></td>
<td>1981</td>
<td>ICMR bulletin, 2008; Bloland, 2001</td>
</tr>
<tr>
<td>Artemisinin</td>
<td><em>P. falciparum</em></td>
<td>-</td>
<td>World malaria report, 2009</td>
</tr>
</tbody>
</table>

Resistance due to the **indiscriminate/inappropriate** use of drugs, **non compliance** and the **close structural relationship** of anti-malarial drugs in use
Main objective of the study

To search for anti-malarial bioactivities of the crude extracts and isolated secondary metabolites from the rhizomes of *Kniphofia foliosa*
Specific objectives of the study

1. To collect, extract and isolate secondary metabolites from the rhizomes of *Kniphofia foliosa*

2. To carry out **structural elucidation** of the isolated secondary metabolites using various spectroscopic methods

3. To establish the **in vitro** anti-plasmodial properties of the crude extracts, and isolated secondary metabolites and compare them with the current anti-malarial drugs

4. Establish the **in vivo** anti-plasmodial properties of the isolated secondary metabolite with good **in vitro** anti-plasmodial activity
JUSTIFICATION
Why the focus on drug development as a solution to the malaria problem?

• Due to the persistent problem of resistance, there is urgent need for new anti-malarial drugs that are both effective and affordable.

• Also in order to delay onset of resistance there is need for a pool of effective anti-malarial drugs with different modes of action.

• To avail cheap and effective herbal drugs
Why the focus on plants as a solution to the existing malaria problem?

Quinine
From *Cinchona succuriba*

Artemisinin
From *Artemisia annua*

Use of natural products with therapeutic properties as ancient as human civilisation with plants being a main source of drugs (Rates, 2001)
Why the focus on plants belonging to the genus *Kniphofia*

*K. foliosa*

*(Wube et al., 2006)*

Some species belonging to the genus *Kniphofia* are used in *ethno-medicine* for the *treatment of malaria*
Why the focus on metabolites belonging to the genus *Kniphofia*

Secondary metabolites with promising anti-plasmodial activity isolated from *Kniphofia foliosa* plant

- **Chrylandicin** 0.54 (3D7 strain)  
  *Wube et al., 2005*

- **Knipholone** 1.06 (K1 strain)  
  *Bringmann et al., 2008a*
Scope of study

- The plant considered for this study was *Kniphofia foliosa*
- The selection of this plant was based on the ethno-medical uses and chemotaxonomic/bioassay information from the available literature (Wube *et al.*, 2006; Bringmann *et al.*, 2008a).
Botanical information on *Asphodelaceae*

Two sub-families
17 genera, 750 species

11 genera, app. 261 species

- **Asphodeloideae**
  - **Kniphofia**
    - 70 species in Africa, 1 in Kenya (*K. thomsonii*)
    - *K. foliosa* perennial herb endemic to Ethiopia
  - **Bulbine**
    - 41 species in Southern Africa, 2 species in Kenya

7 genera

- **Alooideae**
  - **Aloe**
    - app. 400 species, 83 in East Africa.
    - 60 in Kenya
  - **(Smith and Van Wyk, 1991)**
# Ethno-medical information on some species of the genus *Kniphofia*

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant part</th>
<th>Used for/ As</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. foliosa</em></td>
<td>Roots</td>
<td>Abdominal cramps and aches</td>
<td>Wube <em>et al.</em>, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wound healing</td>
<td>Wube <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><em>K. linearifolia</em></td>
<td>Roots</td>
<td>To treat infertility in women</td>
<td>Bosch, 2008</td>
</tr>
<tr>
<td><em>K. isoetifolia</em></td>
<td>Roots</td>
<td>Gonorrhea, hepatitis B</td>
<td>Yineger <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><em>K. laxiflora</em></td>
<td>Plant infusion</td>
<td>Snake deterrents; chest ailments</td>
<td>Ramdhani, 2006; Bringmann <em>et al.</em>, 2008</td>
</tr>
</tbody>
</table>
Some biological activities of metabolites of the genus *Kniphofia*

<table>
<thead>
<tr>
<th>Biological activity</th>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant activity</td>
<td>Knipholone anthrone</td>
<td>Habtemariam, 2007; Bringmann <em>et al.</em>, 2008a</td>
</tr>
<tr>
<td>Antiprotozoal activity, radical scavenging effect</td>
<td>Knipholone anthrone</td>
<td>Habtemariam, 2007</td>
</tr>
<tr>
<td>against DPPH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antitumour activities (HSC-2 cells)</td>
<td>Knipholone isoknipholone</td>
<td>Bringmann <em>et al.</em>, 2008a</td>
</tr>
<tr>
<td>Antimalarial (K1 and NF54 strains of <em>Plasmodium falciparum</em>)</td>
<td>Isoknipholone Knipholone anthrone</td>
<td></td>
</tr>
</tbody>
</table>
Phytochemistry of *Kniphofia*

**Islandicin**
*Berhanu et al.*, 1986

**Chryslandicin**
*Dagne et al.*, 1987

**Knipholone**
*Yenesew et al.*, 1994

**Knipholone cyclooxanthrone**
*Abdissa et al.*, 2013
METHODOLOGY
Overview of Methodology

• **Field collection:** Rhizomes of *K. foliosa* plant collected from the Chemistry Department, Addis Ababa University garden, Ethiopia in November, 2009

• **Extraction:** (solvents used DCM:MeOH (1:1) and MeOH)

• **Isolation and Purification:** (Column chromatography, Sephadex (LH-20), Prep. Thin layer chromatography)

• **Structure elucidation:** (^1H and ^13C NMR, UV, Mass Spectroscopy)

• **Bioassays:** *in vitro* and *in vivo* anti-plasmodial assays (Yenesew *et al.*, 2012) - in collaboration with KEMRI
Plant collection and processing
Extraction and isolation of compounds

Led to the isolation of 14 compounds
Characterization of compounds

At the University of Nairobi

$^1H$ (200 MHz)
$^{13}C$ NMR (50 MHz)

Additional data to be generated:
UV-VIS SPECTRA
IR SPECTRA
MELTING POINT
Biological tests

Anti-plasmodial activity (in vitro)

Antimalarial activity (in vivo)
RESULTS AND DISCUSSION
Compound 1

Physical properties:
• obtained as a pale yellow amorphous solid that intensifies in colour upon exposure to ammonium vapour
• Eluted with 15% EtOAc in n-hexane
• Has an Rf value of 0.3 in 100% dichloromethane
UV data of Compound 1

UV data: ($\lambda_{\text{max}}$ 284, 360 and 412 nm) which is indicative of a phenylanthrone moiety
\(^1\text{H NMR (Expansion) Spectrum Data for Compound 1}\)

\(\delta_H \text{ H-2: 6.98, s}\)

\(\delta_H \text{ H-5: 7.62, t, } J=7.8 \text{ Hz}\)

\(\delta_H \text{ H-6: 7.02, } dd, J=1.2, 7.8 \text{ Hz}\)

\(\delta_H \text{ H-7: 7.45, } dd, J=1.2, 7.8 \text{ Hz}\)

\(\delta_C 147.4\)

\(\delta_H 2.38\)

Suggestive of the presence of a chrysophanol anthrone moiety
$^1$H NMR (Expansion) Spectrum Data for Compound 1

Suggests the presence of an acetylphloroglucinol methyl ether substituent
The $^{13}$C NMR spectrum showed the presence of twenty-seven non-equivalent carbon atoms.

76.7, O-sp$^3$ C
$^1$H NMR (Expansion) Spectrum Data for Compound 1 cont.

- **4.00 s OCH$_3$ - 4’**
- **3.17, d, J=15 Hz**
- **2.87, d, J=15 Hz CH$_2$ - 1’’**
- **3.17, d, J=15 Hz**
- **2.87, d, J=15 Hz CH$_2$ - 1’’**
- **CH$_3$ -3’**
- **CH$_3$ -3’’**
- **CH$_3$ -3’’**
HSQC Spectrum Data for Compound 1

δ_H 3.17, d, δ_H 2.87, d

δ_C 51.1
$^{13}$C NMR Spectrum Data for Compound 1

$\delta_C$ 203.7

$\delta_C$ 204.6 C-3'

$\delta_C$ 192.6 C-9
HMBC Spectrum Data for Compound 1

Correlation between the methylene and methyl protons with the carbonyl at \( \delta_C 203.7 \) confirms the presence of an acetonyl substituent.
Correlation between the methylene protons of the acetonyl substituent and C-10 (δ_c 76.7) confirms the placement of the acetonyl substituent at C-10.
The molecular ion peak at m/z 474.1303 corresponding to molecular formula C_{27}H_{22}O_{8}
The lack of OH signals (except for the chelated hydroxyl groups) even in acid free solvent is evident.
Final structure

By further making reference to Abdissa et al., 2013, compound 1 characterised as 10-acetonylknipholone cyclooxanthrone.
Other compounds isolated from this study

**Anthraquinones**

- **Chrysophanol**
  Berhanu *et al.*, 1986

- **Aloe-emodin acetate**
  Berhanu and Dagne, 1984

- **Deoxyerythrolaccin**
  Yagi *et al.*, 1974

- **Islandicin**
  Berhanu *et al.*, 1986

- **Laccaic acid D**
  DNP, 2009
Other compounds isolated in this study cont.

**Anthraquinone dimers**

- Chryslandicin 10 – methyl ether
- Chryslandicin, *Dagne et al.*, 1987
Other compounds isolated in this study cont.

Phenylanthraquinones/ anthrones

Knipholone
Yenesew et al., 1994

Knipholone anthrone
Yenesew et al., 1994
Other compounds isolated in this study cont.

PhenylInthraquinone dimers

Joziknipholone A
Bringmann et al., 2008b

Joziknipholone B
Bringmann et al., 2008b
Other compounds isolated in this study cont.

Minor phenolic (benzoic acid derivative)

3,4-dihydroxybenzoic acid
Chemotaxonomic significance of this study

• This is only the second report on the occurrence of the dimeric phenylanthraquinones Joziknipholones A and B in nature having previously been isolated from the roots of *Bulbine frutescens* (Bringmann *et al.*, 2008b)

• Deoxyerythrolaccin, laccaic acid D, asphodelin and 3,4-dihydroxybenzoic acid are reported for the first time from the genus
**in-vitro** antiplasmodial activities of crude extracts and selected compounds of the rhizomes of *K. foliosa* against (D6) and (W2) strains of *P. falciparum*

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ values (µg/mL ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D6</td>
</tr>
<tr>
<td><strong>Crude extracts</strong></td>
<td></td>
</tr>
<tr>
<td><em>K. foliosa</em> (DCM:MeOH extract)</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td><em>K. foliosa</em> (MeOH extract)</td>
<td>4.7±0.5</td>
</tr>
<tr>
<td><strong>Dimeric anthraquinones</strong></td>
<td></td>
</tr>
<tr>
<td>Chryslandicin</td>
<td>2.1±0.5</td>
</tr>
<tr>
<td>10-hydroxy-10- (chrysophanol-7’-yl) chrysophanol anthrone</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>Asphodelin</td>
<td>8.2±1.7</td>
</tr>
</tbody>
</table>

**KEY:**
D6: Chloroquine sensitive
W2: Chloroquine resistant

(Yenesew et al., 2012)
**in-vitro** antiplasmodial activities of crude extracts and selected compounds of the rhizomes of *K. foliosa* against (D6) and (W2) strains of *P. falciparum* cont.

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<th>Sample</th>
<th>IC$_{50}$ values (µg/mL ± SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>D6</td>
</tr>
<tr>
<td><em>Phenylanthaquinones/ anthrones</em></td>
<td></td>
</tr>
<tr>
<td>Knipholone</td>
<td>10.1±0.2</td>
</tr>
<tr>
<td>Isoknipholone</td>
<td>8.6±1.6</td>
</tr>
<tr>
<td>Knipholone anthrones</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td>10-Acetonylknipholone cyclooxanthrone</td>
<td>4.4±1.5</td>
</tr>
<tr>
<td><em>Dimeric phenylanthaquinones</em></td>
<td></td>
</tr>
<tr>
<td>Joziknipholone A</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Joziknipholone B</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td><em>Standard drugs</em></td>
<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.007±0.001</td>
</tr>
<tr>
<td>mefloquine</td>
<td>0.03±0.01</td>
</tr>
</tbody>
</table>
*in vivo* antiplasmodial activity for knipholone anthrone isolated from the rhizomes of *Kniphofia foliosa*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose mg/kg/day</th>
<th>Mean (SD) value %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parasitemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemosupression</td>
</tr>
<tr>
<td>Knipholone anthrone</td>
<td>100</td>
<td>18.2 (2.3)</td>
</tr>
<tr>
<td>Quinine hydrochloride</td>
<td>100</td>
<td>0.00</td>
</tr>
</tbody>
</table>

in an *in vivo* 4-day *Plasmodium berghei* ANKA suppressive test at 100 mg/kg/day; knipholone anthrone showed marginal activity with 30.13% chemosupression being observed
Conclusion

• A total of fourteen (14) compounds have been isolated from the rhizomes of *K. foliosa* of which two namely 10 acetonylknipholone cycloanthrone (1) and chryslandicin-10-metyl ether are novel.

• The dimeric phenylanthraquinone Joziknipholone A showed the highest activity with IC$_{50}$ values of 0.4±0.1 and 0.3±0.1 µg/ml against D6 and W2 strains.

• 10 acetonylknipholone cycloanthrone (1) exhibited significant activity with an IC$_{50}$ value of 3.1±1.2 µg/ml against the chloroquine resistant (W2) strain.

• Marginal activity observed for knipholone anthrone (30.13% chemosupression) in an *in vivo* 4-day *P. berghei* ANKA suppressive test at 100 mg/kg/day.
Recommendations/ Further work

• To test the pure enantiomeric forms or at least the enantiomerically enriched forms of the phenyantraquinones in order to establish the relationship between configuration and anti-plasmodial activity

• Further phytochemical work on other species of *Kniphofia*

• To test the isolated compounds against a wider range of micro-organisms (both anti-bacterial and anti-fungal) and other strains of *P. falciparum*
Publications to date


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