Anti-Malarial Activities of *Margaritaria discoidea* and Other Nigerian Medicinal Plants

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OVERVIEW

- Malaria – A Global Night-Mare
- Current Status of Medicinal Plants
- Aims of This Study
- Experiments
- Significant Findings
- Summary
Malaria – A Global Night-Mare

• Malaria is an important tropical communicable disease, which accounts for about 800,000 deaths annually, and exerts a huge burden of about $12 billion on the African economies.

• 198 Million cases of malaria was reported in 2013.

• Presently, about 3.3 billion people in 97 countries are at risk of malaria, with 1.2 Billion people at a higher risk.

• A child dies every minute of malaria in Africa

WHO, WORLD MALARIA REPORT, 2014; WHO, Malaria Fact Sheet No 94, 2015
CURRENT STATUS OF MEDICINAL PLANTS

- Ethno-pharmacology, which is the study of how different ethnic groups use and formulate their drugs has been an important source of medicine.

- The ACT malaria drug-artemisinin was obtained from a medicinal plant *Artemisia annua*

- Currently, the searchlight is on medicinal plants research aimed at obtaining more potent antimalarial in view of the emerging artemisinin resistance.
AIMS OF THE STUDY

- To screen ten Nigerian plant extracts both in aqueous and organic phases for antimalarial activity

- To further investigate the extracts showing high levels of antimalarial activity for possible active compounds

- To modify the obtained active compounds for possible increase in potency and for a SAR investigation
TEN NIGERIAN MEDICINAL PLANTS INVESTIGATED

- Anchomanes difformis
- Bombax buonopozense
- Corchorus olitorus
- Fleurya sp.
- Margaritaria discoidea
- Milletia thonningii
- Moringa oleifera
- Mucuna pruriens
- Newbouldia laevis
- Platycerium bifurcatum
MARGARITARIA DISCOIDEA

- Anti-oxidant, anti-inflammatory and anti-bacterial activities (Dickson et al., 2011).
- Filaricidal activities (Cho-Ngwa et al., 2010).
- In vitro anti-cancer activity on ovarian cancer cell lines (Johnson-Ajinwo et al., 2015)
The extracts were obtained by sequential extraction with organic solvents and water.

Preliminary screening of the extracts to ascertain the promising extracts was carried out.

Bio-assay guided isolation of active compounds by column chromatography from most active extract.

Characterization of the isolates by GC-MS, NMR, and LC-MS.

Determination of the IC$_{50}$ of the isolated/standard compounds.

Preliminary chemical modification of the most active compound.
SCHEME FOR EXTRACTION AND FRACTIONATION PROCEDURES

Pulverized plant materials

Macerate with DCM/MeOH (1:1)

plant residue

Macerate with MeOH

plant residue

Macerate with deionized water

Aqueous extracts

DCM/MeOH extracts

combine

MeOH extracts

Organic extracts

Partition with n-hexane, ethyl acetate, and n-butanol

n-hexane fraction

ethyl acetate fraction

n-butanol fraction

water fraction
**In Vitro Malarial Screening Method**

- The screening was carried out, using red blood cells cultured with the 3D7 strain of *P. falciparum*.

- The antimalarial activity was analysed using the SYBR-green I fluorescence assay method to view the percentage growth of *P. falciparum* at three concentrations of extracts.

- The drugs were added at a two-fold serial dilution, to determine the IC$_{50}$.

- Experiments were conducted in triplicates.
## RESULTS OF SCREENING

<table>
<thead>
<tr>
<th>PLANT</th>
<th>% GROWTH OF PARASITE AT LOW CONC (11.1µG/ML)</th>
<th>% GROWTH OF PARASITE AT MEDIUM CONC (33.3µG/ML)</th>
<th>% GROWTH PARASITE AT HIGH CONC (100µG/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. DISCOIDEA ORG</td>
<td>100</td>
<td>62</td>
<td>7</td>
</tr>
<tr>
<td>M. DISCOIDEA AQ</td>
<td>100</td>
<td>90</td>
<td>58</td>
</tr>
<tr>
<td>N. LAEVIS ORG</td>
<td>95</td>
<td>85</td>
<td>45</td>
</tr>
<tr>
<td>N. LAEVIS AQ</td>
<td>98</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>C. OLITORUS ORG</td>
<td>98</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>C. OLITORUS AQ</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. BIFURCATUM ORG</td>
<td>100</td>
<td>97</td>
<td>70</td>
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<tr>
<td>P. BIFURCATUM AQ</td>
<td>100</td>
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<td>80</td>
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<tr>
<td>A. DIFFORMIS ORG</td>
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<td>70</td>
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<tr>
<td>A. DIFFORMIS AQ</td>
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<td>90</td>
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<tr>
<td>M. OLEIFERA ORG</td>
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<td>40</td>
</tr>
<tr>
<td>M. OLEIFERA AQ</td>
<td>95</td>
<td>82</td>
<td>70</td>
</tr>
<tr>
<td>B. BUONOPOZENSE ORG</td>
<td>90</td>
<td>70</td>
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<tr>
<td>B. BUONOPOZENSE AQ</td>
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<tr>
<td>MILLETIA THONNINGII ORG</td>
<td>98</td>
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<tr>
<td>MILLETIA THONNINGII AQ</td>
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<tr>
<td>MUCUNA PRURIENS ORG</td>
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<td>MUCUNA PRURIENS AQ</td>
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<tr>
<td>FLEURYA SP ORG</td>
<td>90</td>
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<td>45</td>
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<tr>
<td>FLEURYA SP AQ</td>
<td>100</td>
<td>95</td>
<td>60</td>
</tr>
</tbody>
</table>
Graphical representation of partitioned fractions of *M. discoidea*
GC-MS CHROMATOGRAM OF TMS DERIVATIVES OF THE COMPOUNDS IN ETHYL ACETATE FRACTION OF *M. DISCOIDEA*
MASS SPECTRUM SECURINININE (15) IN M. DISCOIDEA (A) AND THAT OF SECURINININE FROM NIST LIBRARY (B)
SOME IDENTIFIED COMPOUNDS IN THE ETHYL ACETATE FRACTION OF *M. DISCOIDEA*
ANTIMALARIAL ACTIVITY OF SOME IDENTIFIED COMPOUNDS

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SECURININE</td>
<td>11.3 ± 0.6</td>
</tr>
<tr>
<td>(+)-CATECHIN</td>
<td>112.3 ± 0.7</td>
</tr>
<tr>
<td>(-)-GALLOCATECHIN</td>
<td>119.9 ± 0.6</td>
</tr>
<tr>
<td>BETULLINIC ACID</td>
<td>&gt;180</td>
</tr>
<tr>
<td>GALLIC ACID</td>
<td>26.8 ± 1.3</td>
</tr>
</tbody>
</table>
$IC_{50}$ CURVE FOR SECURININE
CHEMICAL MODIFICATION OF SECURININE

\[ IC_{50} > 350 \, \mu M \]

The double bond is key to maintain its anti-malarial activity.
SUMMARY

- *Margaritaria discoidea* (org) and *Milletia thonningii* (aq) were the most promising anti-malarial extracts.

- This *in vitro* work supports the traditional use of *M. discoidea* for the treatment of malaria.

- Securinine is the most bioactive compound in *M. discoidea*
FUTURE WORK

• More securinine analogues would be synthesised to improve its potency and for a SAR study.

• To investigate the antimalarial compounds in Milletia thonningii.
APPRECIATION

• Many Thanks to Dr. Paul Horrocks and his team, who conducted the anti-malaria screening of the plant extracts/compounds.

• Dr Wen-Wu Li, my supervisor, who has worked tirelessly in the research project.

• John Clews who did the NMR analysis.
THANKS FOR LISTENING!

ANY QUESTIONS?