G.C – MS ANALYSIS AND IN VIVO ANTIMALARIAL CHEMOTHERAPEUTIC ACTIVITY OF OIL ISOLATED FROM EUPHORBIA HIRTA (CAT’S HAIR) L.

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Abstract
The study was conducted to determine the in vivo antimalarial activity of Euphorbia hirta used by rural dwellers in Edo State, Nigeria. Euphorbia hirta (Cat’s hair) L, Family- Euphorbiaceae, whole plant were collected, dried, pulverised and extracted with methanol solvent in a soxhlet extractor. The extract was concentrated with rotary evaporator and partitioned with methanol: hexane: water (3:6:1). The hexane fraction was isolated with vacuum liquid chromatography (VLC). GC-MS was used to characterised the oil, acute toxicity test was determined to estimate the lethal dose (LD50) value of the extract in balb/c albino mice. Chemo suppressive activities were conducted along with quinine to determine the antimalarial potency of the oil in Plasmodium –infected balb/c albino mice. From the GC-MS results 9-hexadecenal was the major constituent indicated, LD50 of 44.67mg/kg/body weight of mice was interpolated from the linear regression graph and the chemo suppressive activities revealed that the isolated oil exhibit significant suppression (P<0.05) of Plasmodium falciparum which was evident by the 39.18% mean chemo suppression of malaria parasite at day 4 of treatment of P. falciparum –infected mice with 40mg/kg/day. This work corroborates the local use of the plant for the treatment of malaria.

Keywords: Euphorbia hirta, GC-MS, antimalarial, Plasmodium falciparum, in vivo

Introduction
Malaria is the most important parasitic disease in the world and it is widespread in tropical and subtropical regions, including parts of the America, Asia and Africa. Each year, there are approximately 515 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa[1]. In Nigeria, over 100 million people are at risk of malaria every year, and it is estimated that about 50 percent of the adult population experience at least one episode of the disease yearly, while children of under five years old have up to two to four attacks of the disease annually [2]. In Nigeria, Ghana, Mali, and Zambia, the first line of treatment for 60 percent of children with high fever resulting from malaria is the use of herbal medicines at home [3].

Euphorbia hirta (Cat’s hair) is a slender-stemmed, annual hairy plant with many branches from the base to the top, spreading up to 40cm in height [4]. Active constituents, such as afzein, quercitrin, and myricitrin have been isolated from methanol extract of Euphorbia hirta [5]. Some natives in Edo State, particularly the ‘Binis’ and ‘Akoko Edo’, cut the stem and leaves to prepare a concoction for the treatment of fevers and stomach ache arising from malaria[6]. As a result of the increasing spread of drug-resistant Plasmodium falciparum strains, and the continuing threat to Africans by this tropical disease has lends urgency to the need to expand the systematic exploration of medicinal plants in the search of new drugs or its precursor. Thus, the study was conducted to determine the in vivo antimalarial activity of isolated oil of E. hirta.

Materials and Method

Chemicals and Reagents
All chemicals used in this work were of analytical grade from Merck and Sigma Aldrich and they include methanol, n-hexane, dimethyl sulphoxide (DMSO), and ethanol.

Sample Collection and Treatment
The fresh leaves of E. hirta were collected from the bush in Ekiadolor community in Ovia North East Local Government Area of Edo State, Nigeria. The plant was identified by Prof. J.F. Bamidele, a taxonomist in the Department of plant Biology and Biotechnology, University of Benin. The plant leaves were air-dried under shade in the laboratory for four weeks and pulverizer to a powdered form. Extraction was done in soxhlet extractor using methanol as a
isolvent. The crude extract was dried using Na$_2$SO$_4$ and concentrated in a rotary evaporator.

**Isolation of Oil**

60g of methanol extract was partitioned with 100ml of hexane: methanol: water mixture (ratio: 3:6; 1) and shaken vigorously in a separatory funnel. The upper hexane fraction was separated, concentrated and then subjected to vacuum liquid chromatography (VLC), using silica gel (particle size: 200-425 mesh) as the solid phase and 100% hexane as the mobile phase. A yellow oily phase obtained was dried over Na$_2$SO$_4$ and concentrated to recover the pure oil (4.7ml by volume; yield 7.83%) labeled as EHHVLC1 (E.hirta oil)

**GC-MS Analysis**

The analysis was carried out on a GC -MS spectrometer filled with an HP-5 MS (5% phenylsiloxane) column at a temperature programme of 70°C (2 minutes) increase at 10°C/min to 280°C and held for 7minutes. The carrier gas was nitrogen and flow rate, 1.80mL/min. The GC-MS analysis was done on a Shimadzu, GC-MS QP2010 at NARICT, Zaria.

**Acute toxicity test**

The acute toxicity assay was performed according to the procedure described by Lorke [7] to estimate the LD$_{50}$ values of the isolated oil of E.hirta using Balb/c albino mice before the antimalarial analysis (using P. falciparum-infected mice).

**Chemo suppressive activities (Antimalarial chemotherapeutic) Development of experimental humanized mouse model**

Mice: Balb/c mice weighing 20 – 33 g were used. They were housed in standard mosquito-netted metal cages under standard conditions of light and temperature and were maintained on a standard mice diet and water ad libitum. They were acclimatized for 14 days and were treated in accordance with guidelines for animal care approved by the Animal Ethics Committee of the University of Benin, Benin City, Nigeria. The mice were certified medically fit for the experiment by Dr. J. Danjuma, a veterinary Doctor.

Modification of mice: The Balb/c mice were modified by using pharmacological compounds (4mg aspirin/kg body weight and 4mg anhydrous doxycycline eq./kg body weight) to retard their innate immune responses [8],[9],[10] followed by engraftment of human blood [10] and [11]. Infection of experimental humanized mouse model with Plasmodium falciparum were done according to the method of Trager and Jensen and Lambros and J.Vanderberd [12],[13].

Antimalarial chemotherapy of the P. falciparum-infected mouse model with isolated oil of E. hirta

P. falciparum-infected humanized immunosuppressed mice were employed to verify the antimalarial principles of each of the test samples. The mice were divided into 5 independent experimental groups (5 mice per group). The negative control drug –DMSO (4mg/kg body weight administered three time daily for four days) and positive control drug- quinine (73mg/kg body weight administered three times daily for four days) were used to treat mice in two of the experimental groups (antimalarial validation). The mice received these drugs orally via a gastric cannular. Varying doses (10, 20 and 40 mg/kg/day each) of the test oil were used to treat the mice in the other three experimental groups. The test samples were administered, by taking into consideration their LD$_{50}$ values.

Upon administration of quinine, DMSO, and the plant oil; parasite densities, haematocrit, as well as total leukocyte counts, were obtained from mice that either died or survived during the chemotherapy phase, according to the methods stated by [14].Net mean chemo suppression [15] due to drug/extracts administration was calculated according to the formula:

\[
\text{Net mean chemo suppression (\%)} = \frac{100 \times (A-B)}{A} - MC0
\]

Where,

A = Percentage of parasitaemia in the mice administered with DMSO.
B = Percentage of parasitaemia in mice treated with quinine/plant extracts.

\[
\frac{100 \times (A-B)}{A} = \text{Mean chemo suppression}
\]

MC0 = Mean chemo suppression obtained from mice at day 0 of treatment.

**Statistical analysis**

Values were expressed as mean and standard error of the mean, as well as in percentages. Chi-square test and regression analysis, where appropriate, were used to determine the level of significance, and P-value less than 0.05 (P < 0.05) were considered significant. The software, SPSS version 16, was employed for the statistical analysis.

**Results and Discussion**

The chemical compounds identified in the oil fraction are presented in Table 1.
Table 1: GC-MS Analysis of isolated yellow oil of *E. hirta*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Retention Time (Rt)</th>
<th>Name of compound</th>
<th>Area percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4</td>
<td>1,1,3-trimethyl cyclogeranniolane</td>
<td>1.60</td>
</tr>
<tr>
<td>2</td>
<td>4.7</td>
<td>Tridecan-1-ol</td>
<td>1.92</td>
</tr>
<tr>
<td>3</td>
<td>5.3</td>
<td>Dimethyl-2-octene</td>
<td>1.78</td>
</tr>
<tr>
<td>4</td>
<td>5.9</td>
<td>2-isopropyl-5-methyl cyclohexyl-2-butenoate</td>
<td>1.80</td>
</tr>
<tr>
<td>5</td>
<td>14.6</td>
<td>2-methyl decane</td>
<td>1.70</td>
</tr>
<tr>
<td>6</td>
<td>19.4</td>
<td>7-hexadecenoic acid</td>
<td>2.10</td>
</tr>
<tr>
<td>7</td>
<td>20.0</td>
<td>9-hexadecenal</td>
<td>12.29</td>
</tr>
<tr>
<td>8</td>
<td>22.6</td>
<td>8,10-hexadecadien-1-ol</td>
<td>5.49</td>
</tr>
</tbody>
</table>

From Table 1, eight major components with abundance of 1% of total and above are reported. The retention times (Rt) are in minutes.

From the toxicity results, the minimum dosage of the isolated oil sample that kills 50% of experimental animals (in this work, balb/c albino mice) was 44.67 mg/kg body weight.

### Antimalarial Chemotherapy

Table 2: Chemosuppressive activities of an isolated compound obtained from *E. hirta* extract against *P. falciparum*

<table>
<thead>
<tr>
<th>Days of Therapy</th>
<th>Experimental Group of mice</th>
<th>Dose of therapeutic agents given</th>
<th>Mean parasite counts ((\times10^5 \text{ cells/\mu l}))</th>
<th>MCS (%)</th>
<th>NCS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>NC group</td>
<td>12mg/kg/day</td>
<td>77.05±3.18</td>
<td>1.54±0.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PC group</td>
<td>219mg/kg/day</td>
<td>76.14±4.45</td>
<td>1.52±0.09</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>T1 EHHVLC1</td>
<td>10mg/kg/day</td>
<td>75.85±2.17</td>
<td>1.52±0.04</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>T2 EHHVLC1</td>
<td>20mg/kg/day</td>
<td>76.19±0.84</td>
<td>1.52±0.02</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>T3 EHHVLC1</td>
<td>40mg/kg/day</td>
<td>77.07±1.99</td>
<td>1.60±0.03</td>
<td>{0.03}</td>
</tr>
<tr>
<td>2</td>
<td>NC group</td>
<td>12mg/kg/day</td>
<td>80.79±2.60</td>
<td>1.60±0.05</td>
<td>60.67</td>
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<tr>
<td></td>
<td>PC group</td>
<td>219mg/kg/day</td>
<td>31.78±1.28</td>
<td>0.6±0.03</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T1 EHHVLC1</td>
<td>10mg/kg/day</td>
<td>79.85±5.60</td>
<td>1.60±0.11</td>
<td>1.17</td>
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<tr>
<td></td>
<td>T2 EHHVLC1</td>
<td>20mg/kg/day</td>
<td>70.46±0.29</td>
<td>1.41±0.01</td>
<td>12.80</td>
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<tr>
<td></td>
<td>T3 EHHVLC1</td>
<td>40mg/kg/day</td>
<td>74.43±2.73</td>
<td>1.49±0.06</td>
<td>7.88</td>
</tr>
<tr>
<td>4</td>
<td>NC group</td>
<td>12mg/kg/day</td>
<td>83.58±15.94</td>
<td>1.67±0.32</td>
<td>83.82</td>
</tr>
<tr>
<td></td>
<td>PC group</td>
<td>219mg/kg/day</td>
<td>13.52±0.57</td>
<td>0.27±0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T1 EHHVLC1</td>
<td>10mg/kg/day</td>
<td>70.83±2.10</td>
<td>1.42±0.04</td>
<td>15.26</td>
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<tr>
<td></td>
<td>T2 EHHVLC1</td>
<td>20mg/kg/day</td>
<td>53.26±4.04</td>
<td>1.06±0.08</td>
<td>36.35</td>
</tr>
<tr>
<td></td>
<td>T3 EHHVLC1</td>
<td>40mg/kg/day</td>
<td>50.84±1.37</td>
<td>1.02±0.03</td>
<td>39.18</td>
</tr>
</tbody>
</table>

MCS = mean chemosuppression; NCS = net mean chemosuppression; NC = negative control group of *P. falciparum*-infected mice which received dimethyl sulfoxide; PC = positive control group of *P. falciparum*-infected mice which were administered with quinine; T1 EHHVLC1, T2 EHHVLC1, and T3 EHHVLC1 = the three experimental groups of *P. falciparum*-infected mice which were administered with varying doses of *E. hirta*-derived isolated oil.

Table 2 represents the chemo suppressive activities obtained during therapy of *P. falciparum*-infected mice with the test sample (EHHVLC1) and positive...
control (quinine). Quinine-treated mice had the lowest parasite counts (mean parasite counts at day 4 of therapy) with 0.27±0.01%, while the lowest parasite count for EHHVLCl was recorded as 1.02±0.03 (day 4, 40mg/kg/day) and in the mice treated with DMSO as 1.67±0.32%, thus indicating that the oil has suppressive effect on \textit{P. falciparum} parasite more than the negative control group but less than quinine treated group. Mean chemo suppression of quinine treated \textit{P. falciparum}—infected mice increased successively from day 0, 2 and 4 with 1.18%,60.67% and 83.82% respectively while the isolated oil at day 0, 2 and 4 (with 40mg/kg/day dose) had mean chemo suppression of 0%,7.88% and 39.18% respectively. All the doses of the isolated compound (EHHVLC 1) exhibited significant suppression (P < 0.05), except the 10 mg /kg/day dose which showed non-significant suppression (P >0.05) when compared to parasite suppression exhibited by quinine.

**Conclusions**

Our study suggests that at high dosages, oil of \textit{E. hirta} contain antimalarial principles that result in significant apoptosis of \textit{Plasmodium falciparum} parasites. However, the use of these extracts at dosages that are comparable to the dose employed in quinine therapy is limited by their relatively high toxicity, as indicated by their LD\textsubscript{50} values. Therefore, there is a need for a suitable detoxification protocol which can significantly inactivate the toxic constituents in these plants to be developed, to enable their extracts to be used at high dosages for therapeutic purposes. In addition, the development of a novel protocol that can facilitate the isolation and concentration of the active antimalarial principles in these plant extracts can also enormously enhance the usage of these extracts at therapeutic doses which can result in significant apoptosis of \textit{Plasmodium falciparum} parasites.

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**References**