Nutriphytochemical Profiling and Changes in Cardiovascular Risk Index in Malarial Infected Mice Treated with Ethanol Root Extract of Phyllanthus Amarus

Adetunji O. Opajobi¹, Ugochukwu E. Uzuegbu², Ajala Michael Olawale³, Pere-Ebi Y. Toloya⁴, Chinwendu O. Elu⁵, Ikechukwu Igwenyi ⁶, Innocent Onyesom⁷

¹,²,⁴,⁵,⁷Department of Medical biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, P.O. Box 01, Abraka, Delta State, Nigeria; E-mail: aopjobi@delsu.edu.ng

³Department of Chemical Pathology and Metabolic Medicine, Eko University of Medical and Health Sciences, P.M.B. 2009, Ijanikin, Lagos State, Nigeria.

⁶Department of Biochemistry, Faculty of Life Sciences, Ebonyi State University, P. M. B. 53 Abakaliki, Ebonyi Atate, Nigeria.

Abstracts: Purpose: The use of medicinal plants in malarial treatment has become popular in Nigeria, an endemic country, due to failing chemotherapy and high cost of antimalarial drugs. One of such commonly used herbs is Phyllanthus amarus. The antimalarial activity of P. amarus has been consistently reported, giving credence to its traditional use. Nevertheless, effects of such treatment on reducing cardiovascular risk indices is yet to be fully documented. This is imperative since certain metabolic complications like increased plasma lipids have been associated with considerable risks of fatty liver and cardiovascular diseases in severe cases of malaria in humans. Methods: In this present study, therefore, the antiplasmodial action of the ethanol root extract of P. amarus and its ED50 dose were determined and associated cardiovascular risk indices were evaluated in BALB/c malarial infected mice, having ascertained the nutritive quality and phytochemicals of plant root. Results: Results showed that P. amarus root is abundant in alkaloid phytochemical and the roots extracts demonstrated significant antiplasmodial activity in a dose-dependent trend with optimal ED50 and maximal potency in reducing cardiovascular risk indices (Atherogenic index of plasma, AIP and heart disease risk, HDR) and fatty liver, which compared well with the values obtained from the uninfected control mice. Conclusion: The roots of P. amarus possess a wide array of nutrients and phytochemicals, with significant antimalarial activity. The roots also reduced the risks of cardiovascular diseases associated with severe malaria, in experimental mice.

Keywords: Heart Disease Risk Factors, Atherosclerosis, Lipids, Malaria, Biomarkers.

1. INTRODUCTION

Malaria, an oxidative infection, has been reported to alter body function. Changes in plasma and liver lipids concentrations have been reported in acute malarial infection in humans, due to demand of parasite for lipids. The high demand of parasite for lipids, results in dyslipidemia which is characterized by high lipid concentrations[1]. This could subsequently lead to an increased risk of cardiovascular diseases [2]. Lipid profiling are important in cardiovascular disease prevention and therapy[3]. Several studies have reported a positive correlation between infectious diseases and cardiovascular diseases[2]. Although, cardiovascular disease due to malaria is rare, it has been reported in severe cases[4] and as a long term manifestations after treatments[5].

Great progress have been made towards malaria eradication globally, however, the burden of malaria still persists. Yearly morbidity and mortality world wide still records an estimated 228 million cases and 405 thousand deaths[6] The burden is maximally felt in low and middle income countries, where a large percentage of cardiovascular deaths occur[7]. Cardiovascular diseases associated with malaria are often found in Plasmodium falciparum infected patients, which causes the largest burden of disease[8].

Many modern drugs are of plant origin. Man has always used herbs for the treatment of many diseases[9]. One of these herbs commonly used, especially in traditional medicine, is Phyllanthus amarus. P. amarus is usually used as infusion and drunk by Nigerians for health maintenance and it is considered as a wonder-working herb and has
great economic importance. Therefore, this research aimed at determining the phytochemical and nutritive properties of this medicinal plant and evaluate its associated effects on plasma and liver lipid profiles in *Plasmodium berghei* infected mice, while assessing its antiplasmodial activity.

**2. MATERIALS AND METHODS**

**2.1. Collection of Plant Materials**

Whole plants of *Phyllanthus amarus* were collected and roots were separated from whole plant. The root samples were thoroughly washed in clean water, air dried at laboratory room temperature for two weeks, ground into fine powder with laboratory blender. Powdered root sample was stored in an air-tight container for use.

**2.2. Phytochemical Quantification**

Quantification of alkaloids[10], anthraquinones[10], glycoside[10], saponins[11], phenols[12], flavonoids[10], terpenoids[10], steroids[13], phlobotannins[14] and tannins[15] were carried out according to previously described methods.

**2.3. Proximate Analysis and Nutrient Quantification**

*Proximate analysis:* Proximate composition of moisture, crude fibre, crude protein, carbohydrate, caloric value, lipid content, crude fat and ash were estimated by standard methods of the Association of Official Analytical Chemists[16].

*Estimation of Vitamins, Mineral and Micronutrients:* Concentrations of vitamins (thiamine, niacin, riboflavin, ascorbic acid, vitamin E and vitamin A), minerals (calcium, potassium, magnesium, phosphorus, chromium and sodium) and micronutrients (zinc, copper, manganese and iron) were determined according to standard procedures of the Association of Official Analytical Chemists[16].

**2.4. Preparation of Ethanol Root Extract**

Dried powdered root of *Phyllanthus amarus* (about 100g) was extracted with ethanol in soxhlet apparatus for 72 h. Mixture was filtered and concentrated under vacuum. The concentrated filtrate was suspended in distilled water to obtain ethanol extract [17].

**2.5. Experimental Animals, Parasites and Inoculation**

Adult (eight weeks old) Swiss albino male mice (BALB/c albino strain) weighing between 22-28 g b.wt, were procured from the Laboratory Animal Centre, Faculty of Medical Sciences, Delta State University, Abraka. Donor mice already inoculated with *Plasmodium berghei* (Strain NK65) parasites were bought from the Department of Parasitology, Nigerian Institute of Medical Research, NIMR, Yaba, Lagos State, Nigeria. A total of thirty (30) mice selected for this study were grouped into six (6); five (5) mice per group. Animals were segregated into groups as follows:

- **Group 1:** Positive Control (neither infected nor treated),
- **Group 2:** Negative Control (infected, but not treated),
- **Group 3:** Standard Control (infected and treated with 20 mg/kg of standard drug, artemether and lumefantrine, Lonart®DS),
- **Group 4:** Infected and treated with 150 mg/kg of *Phyllanthus amarus* ethanol root extracts (PAERE),
Group 5: Infected and treated with 300 mg/kg of PAERE,

Group 6: Infected and treated with 450 mg/kg of PAERE.

Dosage was selected based on previous studies [18].

Experimental mice were infected with inoculum prepared by diluting 0.1 mL of infected blood obtained from donor mice, in 0.9 mL of PBS, pH 7.2.

2.6. Antimalarial Activity

After inoculation of experimental mice and parasite confirmation (72 h post infection), *P. amarus* ethanol extract and standard drug were administered to test and standard control groups, once daily for four consecutive days via intragastric cannula. Parasitaemia was assessed at day 0, 3, 4, 5 and 6. Parasitaemia was estimated by using blood collected from cut tail tip of infected mice, which were used to make blood smears on microscopic slides. Smears were stained with Giemsa and viewed under microscope at x40 magnification [18]. Percentage parasitaemia, inhibition and growth were calculated using the following formulae:

\[
\% \text{ parasitaemia} = \frac{\text{Number of parasitized red blood cell}}{\text{Total number of red blood cell}} \times 100
\]

\[
\% \text{ inhibition} = 100 - \frac{Pt}{Pl} \times 100
\]

where \(Pt\) = parasitaemia in treatment groups and \(Pl\) = Parasitaemia in negative control

\[
\% \text{ Growth} = \frac{P (d4 - d3) + (d5 - d4) + (d6 - d5)}{n - 1} \times 100
\]

where \(P(d)\) = % parasitaemia at day (d), \(n\) = total days [19].

2.7. Lipid Profiling of Serum And Liver

On day 6, after mice were fasted overnight, about 5ml of blood was collected from ketamine/xylazine euthanatized mice by cardiac puncture with a sterile syringe and needle into plain screw-cap sample bottles and allowed to clot for 2 h and then centrifuged at 3,000 xg for ten minutes at room temperature to obtain the sera. The sera samples were collected by aspiration using a pasture pipette into sterile plain bottles.

Liver from euthanatized mice was collected and processed as required for analysis. A gram of the fresh tissue was blended and homogenized in 49ml of normal saline. The homogenate was then centrifuged (80D Serico, China) at 12000 xg for 15min to obtain the supernatant. Resulting supernatant was collected into plain screw-cap bottle and kept frozen until required for analysis.

Total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein (LDL-C) cholesterol, and high-density lipoprotein (HDL-C) cholesterol were determined using commercial kits (Randox Laboratory Ltd., UK) following the manufacturer’s instruction.

Atherogenic index of plasma (AIP) and heart disease risk (HDR) was calculated with the following formula [20]:

\[
AIP = \log \frac{TAG}{HDL - c}
\]
2.8. Statistical Analysis

Results are displayed as Mean ± SD. Mean of the various groups were compared by using One-Way Analysis of Variance (ANOVA) and Tukey’s multiple comparisons on Microsoft Excel (2013). Results were considered significant at $p < 0.05$.

3. RESULTS

Phytochemical profiling of ethanol root extract of *Phyllanthus amarus* is tabulated in Table 1. Phytochemical screening revealed the presence of a wide array of phytochemicals; alkaloids, anthraquinones, glycoside, saponins, phenols, flavonoids, terpenoids, steroids, phlobotannins and tannins, listed in descending order in relation to their abundance (Table 1).

Ethanol is a better solvent for extraction, judging by results in Table 1. Significant differences ($p<0.05$) existed between percentage phytochemical concentrations in aqueous and ethanol extracts. Although tannin was more present in aqueous extracts, ethanol extracts were selected for further study as they contained higher concentrations of other phytochemicals.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids, %</td>
<td>24.21±0.32</td>
<td>1.55±0.423</td>
<td>3.09E-05</td>
</tr>
<tr>
<td>Flavonoid, %</td>
<td>16.18±0.13</td>
<td>17.35±0.01</td>
<td>0.001744</td>
</tr>
<tr>
<td>Saponin, %</td>
<td>15.21±0.20</td>
<td>20.51±0.21</td>
<td>5.93E-07</td>
</tr>
<tr>
<td>Tannin, %</td>
<td>16.21±0.14</td>
<td>9.10±0.11</td>
<td>2.97E-06</td>
</tr>
<tr>
<td>Glycoside, %</td>
<td>16.10±0.20</td>
<td>26.01±0.06</td>
<td>3.33E-05</td>
</tr>
<tr>
<td>Anthraquinone, %</td>
<td>20.20±0.10</td>
<td>26.60±0.07</td>
<td>7.97E-05</td>
</tr>
<tr>
<td>Steroids, %</td>
<td>12.00±0.23</td>
<td>17.00±0.11</td>
<td>0.000131</td>
</tr>
<tr>
<td>Phlobotannin, %</td>
<td>7.03±0.07</td>
<td>12.33±0.31</td>
<td>0.000116</td>
</tr>
<tr>
<td>Terpenoids, %</td>
<td>0.00±0.00</td>
<td>17.23±0.20</td>
<td>1.1E-05</td>
</tr>
<tr>
<td>Phenols, %</td>
<td>14.02±0.21</td>
<td>19.98±0.03</td>
<td>9.19E-05</td>
</tr>
</tbody>
</table>

*Values are Mean ±SD for triplicate determinations.*

Proximate analysis and nutrient quality of the root extracts are listed in Table 2 and 3, respectively.

Proximate analysis shows high carbohydrate concentrations, with moderate moisture and crude fat concentrations and low concentrations of crude fibre, protein and ash content (Table 2).
The most abundant vitamin in *Phyllanthus amarus* root is vitamin E, with the least, niacin. The roots of *P. amarus* showed low concentrations of minerals. However, the concentrations magnesium and sodium were maximum (Table 3). Our study revealed, iron as the most abundant micromineral in the root of *P. amarus* (127.42 mg/100g). Other micronutrients, zinc and manganese were found in moderate concentrations (33.30 and 36.53 mg/100g, respectively), while copper was found in low concentrations (5.01 mg/100g).

### Table 2. Proximate analysis of *Phyllanthus amarus*

<table>
<thead>
<tr>
<th>Proximate</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>9.75±1.70</td>
</tr>
<tr>
<td>Crude fibre, %</td>
<td>5.00±1.80</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>6.90±1.74</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>80.00±6.81</td>
</tr>
<tr>
<td>Caloric value, kCal/100g</td>
<td>515.67±49.82</td>
</tr>
<tr>
<td>Lipid content %</td>
<td>6.33±1.63</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>13.69±2.75</td>
</tr>
<tr>
<td>Ash content, %</td>
<td>7.43±2.12</td>
</tr>
</tbody>
</table>

*Values are Mean ±SD for triplicate determinations*

### Table 3. Determination of nutrient quality of *Phyllanthus amarus*

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Thiamine, g/100g</td>
<td>16.51±1.67</td>
</tr>
<tr>
<td>Riboflavin, g/100</td>
<td>11.39±2.16</td>
</tr>
<tr>
<td>Ascorbic Acid, g/100g</td>
<td>37.89±3.84</td>
</tr>
<tr>
<td>Vitamin E, Meq/100g</td>
<td>87.72±15.06</td>
</tr>
<tr>
<td>Vitamin A, Meq/100g</td>
<td>46.00±6.48</td>
</tr>
<tr>
<td>Minerals, mg/100g</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0.55±0.17</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.55±0.23</td>
</tr>
<tr>
<td>Magnesium</td>
<td>48.03±4.56</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.44±0.06</td>
</tr>
<tr>
<td>Sodium</td>
<td>109.86±11.92</td>
</tr>
<tr>
<td>Micronutrients, mg/100g</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>33.30±3.17</td>
</tr>
<tr>
<td>Copper</td>
<td>5.01±1.64</td>
</tr>
<tr>
<td>Manganese</td>
<td>36.52±15.92</td>
</tr>
<tr>
<td>Iron</td>
<td>127.42±5.12</td>
</tr>
</tbody>
</table>
Values are Mean ±SD for triplicate determinations.

Daily parasitaemia estimation of post infection and treatments, are illustrated in Figure 1. Parasitaemia reduction following treatments was recorded in Standard Control Group (group treated with Lonart®DS), followed by Group 6 mice (mice treated with 450 mg/kg Phyllanthus amarus ethanol root extract). Parasitaemia reduction in these groups were dose dependent.

This is also reflected in percentage parasite growth and inhibition (Table 4). Percentage growth and inhibition of Group 3 and Group 6 mice presented similar indices. However, ED₅₀ for P. amarus root extract (263.02 mg/kg) were greater than that of standard drug (10 mg/kg).

![Figure 1: Daily records of parasitaemia in malarial infected mice treated with methanol root extract of Phyllanthus amarus. Day 0: Inoculation, Day 3: Confirmation of parasitaemia after 3 days (72h) of post infection. The 4-day treatment period at Day 3, 4, 5 & 6.](image)

Table 4. Percentage of parasite growth and inhibition in P. berghei (NK 65) infected mice at the end of the 4-day treatment period with ethanol root extract of P. amarus

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>Growth, %</th>
<th>Inhibition, %</th>
<th>ED₅₀, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, mL/kg</td>
<td>10</td>
<td>4.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lonart®DS, mg/kg</td>
<td>20</td>
<td>0.2</td>
<td>72.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Ethanol root extract, mg/kg</td>
<td>150</td>
<td>2.3</td>
<td>41.7</td>
<td>263.02</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.5</td>
<td>52.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.3</td>
<td>66.7</td>
<td></td>
</tr>
</tbody>
</table>

ED₅₀, Inhibitory concentration at 50 % parasite inhibition.

Lipid profiles in serum and liver of experimental mice are displayed in Table 5 and 6, respectively. Judging by results displayed in Table 5, following Plasmodium berghei infection, there was significantly (P<0.05) increased concentrations of total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C) and reduced concentrations of high density lipoprotein cholesterol (HDL-C) in serum. The atherogenic index (AIP) and heart disease risk (HDR) in negative control group were also significantly increased, comparing indices in negative control group with the positive control group.

These adverse effects were ameliorated by administration of P. amarus ethanol root extract and standard drug. The amelioration was dose dependent and compared well with the positive control as evidenced by AIP and HDR indices in these groups (Table 5). Serum cholesterol concentrations were reduced in all treatment groups. All groups treated with P. amarus ethanol root extract, showed statistically similar activity (P>0.05), and comparing
indices with positive control groups, reduction in test groups were statistically lower than positive control and standard control ($P<0.05$).

Reduction of TAG and HDL-C followed similar trend. Mice in all test groups displayed statistically similar concentrations ($P>0.05$) with positive and standard control groups.

Changes in LDL-C following infection and treatment with extract were drastic. Great significant reduction was recorded in comparison with negative, positive and standard control groups.

Lower AIP and HDR values signify lower risk of atherogenic complications and heart disease, respectively. All test groups showed statistically significant comparable AIP with positive control groups, however, minimal index was displayed by group 6 mice and this was statistically similar with standard control. Lowest HDR index was exhibited by group 6 mice and this was significantly lower than other groups, including positive control.

### Table 5. Lipid levels (mg/dl) in serum and associated cardiovascular disease risk indices (AIP & HDR) in malarial infected mice treated with ethanol root extract of *Phyllanthus amarus*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose, mg/kg</th>
<th>Cholesterol</th>
<th>Triacylglycerol</th>
<th>HDL-Cholesterol</th>
<th>LDL-Cholesterol</th>
<th>AIP</th>
<th>HDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (NINT)</td>
<td>10</td>
<td>128.33±9.85$^a$</td>
<td>135.00±2.93$^a$</td>
<td>60.00±4.04$^a$</td>
<td>50.50±5.80$^a$</td>
<td>0.35±0.06$^a$</td>
<td>2.15±0.26$^a$</td>
</tr>
<tr>
<td>Water (INT)</td>
<td>10</td>
<td>246.33±4.84$^b$</td>
<td>226.66±4.23$^b$</td>
<td>20.66±4.13$^b$</td>
<td>180.50±6.58$^b$</td>
<td>1.04±0.06$^b$</td>
<td>12.35±2.66$^b$</td>
</tr>
<tr>
<td>Lonart®DS</td>
<td>20</td>
<td>139.66±6.53$^d$</td>
<td>134.66±3.92$^d$</td>
<td>67.66±1.03$^d$</td>
<td>45.03±7.43$^d$</td>
<td>0.29±0.04$^d$</td>
<td>2.06±0.07$^d$</td>
</tr>
<tr>
<td>PAERE 1</td>
<td>150</td>
<td>98.33±2.06$^c$</td>
<td>142.00±2.91$^a$</td>
<td>59.16±7.9$^a$</td>
<td>10.20±6.82$^a$</td>
<td>0.38±0.09$^a$</td>
<td>1.69±0.28$^a$</td>
</tr>
<tr>
<td>PAERE 2</td>
<td>300</td>
<td>96.66±3.72$^c$</td>
<td>138.33±7.08$^a$</td>
<td>60.66±7.00$^a$</td>
<td>8.33±3.83$^c$</td>
<td>0.36±0.07$^c$</td>
<td>1.60±0.15$^c$</td>
</tr>
<tr>
<td>PAERE 3</td>
<td>450</td>
<td>98.33±3.38$^c$</td>
<td>133.66±3.01$^a$</td>
<td>64.16±3.86$^a$</td>
<td>7.43±3.83$^c$</td>
<td>0.31±0.03$^c$</td>
<td>1.53±0.09$^c$</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD for n=5 mice per group. Values bearing different letter superscript in column are significantly different ($p<0.05$). PAERE, *Phyllanthus amarus* ethanol root extract; NINT, Not infected, not treated; INT, infected not treated; AIP, Atherogenic index of plasma = log(TAG/HDL-C); HDR, heart disease risk= TC/HDL-C. The higher the HDR, the more risk. AIP<0.11 low risk, 0.11-0.21 moderate or intermediate risk, >0.21 increased risk for humans[21]

All parameters, TC, TAG, HDL-C and LDL-C, were significantly ($P<0.05$) increased in liver following malaria infection (Table 6). Administration of standard drug and *P. amarus* ethanol root extract significantly decreased these indices.

Improved cholesterol concentrations in test groups was best for Group 4 mice, and this activity was similar with standard control group ($P>0.05$) but significantly higher in comparison with positive control. TAG concentrations in all test groups showed significantly lower ($P<0.05$) values in comparison with standard control which were all significantly higher than positive control, except Group 6 concentrations. Statistically comparable HDL-C with positive control was only achieved by Standard Control and Group 3 mice. All test groups concentrations of LDL-C displayed statistically similar values, but these concentrations were significantly higher than positive and standard control groups.
Table 6. Concentrations of lipids (mg/dl) in liver of malarial infected mice treated with ethanol root extract of Phyllanthus amarus

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose, mg/kg</th>
<th>Cholesterol</th>
<th>Triacylglycerol</th>
<th>HDL-Cholesterol</th>
<th>LDL-Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (NINT)</td>
<td>10</td>
<td>123.66±4.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159.66±5.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.33±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.4±7.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water (INT)</td>
<td>10</td>
<td>232.00±2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>182.66±7.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.33±3.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103.13±7.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lonart®DS</td>
<td>20</td>
<td>140.66±2.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>177.33±3.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.33±3.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.86±6.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAERE 1</td>
<td>150</td>
<td>137.33±2.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>140.66±6.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.33±2.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.86±5.07&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAERE 2</td>
<td>300</td>
<td>150.33±2.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>141.00±7.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.00±6.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.13±7.23&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAERE 3</td>
<td>450</td>
<td>154.33±5.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>153.66±8.82&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55.66±4.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.93±6.25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SD for n=5 mice per group. Values bearing different letter superscript in column are significantly different (p<0.05). PAERE, Phyllanthus amarus ethanol root extract; NINT, Not infected, not treated; INT, infected not treated.

4. DISCUSSION

Traditional plants have a major role in the development of modern medicine [9]. Medicinal plants notable for their healing properties have been confirmed by several researchers and one of these plants is the Phyllanthus amarus. Different parts of the plant have been reported for specific properties, and one of these properties is the antimalarial activity. Antimalarial activity of this plant have been reported in both in vivo and in vitro systems [17,18,22,23]. This antimalarial activity can be attributed to the vast array of nutrients and active biochemical compounds present in the plant [15,23,24]. Therefore, this study was aimed at assessing the nutritional and phytochemical composition of the ethanol root extract of Phyllanthus amarus and its effects on plasma and liver lipid indices in Plasmodium berghei infected mice.

Our study further confirms the positive impact of P. amarus on malarial infection. This could be as a result of the presence of abundant alkaloids in the extract, as alkaloids have been reported severally to possess significant antimalarial properties [25]. P. amarus antimalarial activity has been attributed to its efficiency in stabilizing erythrocyte membrane and inhibiting haem conversion to haemozoin [23]. We also confirmed the presence of high concentrations of glycoside, saponins, anthraquinones, phenols, trepenoid, flavonoids and steroids. Terpenoids have been reported to be the most prevalent phytochemical of the genus of Phyllanthus [26], but alkaloids are the most abundant in Phyllanthus amarus [18,27]. Saponins are said to have antioxidant properties [28]. This could also contribute to antimalarial activity of the extract in our study, as malaria infection induces oxidative stress in host [29]. The presence of phenols and flavonoids in the extract can help prevent oxidative stress by scavenging free radicals [30]. Flavonoids of P. amarus have also been reported to reduce the risk of cerebral malaria by reducing oxidative stress [18]. Minimal amounts of tannins and phlobotannins were also reported.

Proximate analysis revealed low moisture, fibre, protein, ash and lipid content, with high concentrations of carbohydrates and high caloric value. This implies that ethanol extracts provide high energy sources and have a longer shelf life. Plants with low moisture content have a longer shelf life. Studies have shown that crude fibre aids in reducing blood glucose [31]. The low crude fibre content of this plant is, therefore, beneficial in glucose and fat absorption. The most abundant nutrients present were vitamin E, vitamin A, vitamin C, sodium, magnesium, manganese, zinc and iron. High iron content of the roots may be associated with its antimalarial activity as iron supplements have been shown to be beneficial in anaemic conditions like malaria [32]. Also, compounds containing manganese are used as tonic in treating anaemia [33].

Lipid profile of serum of malarial infected mice displayed dyslipidaemia characterized by elevated levels of total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C) and reduced levels of high
density lipoprotein cholesterol (HDL-C). This was accompanied by a high atherogenic and heart disease risk indices. This indicates a high risk of cardiovascular diseases. Liver lipid profiling revealed elevated levels of TC, TAG, HDL-C and LDL-C. Ethanol root extract of *P. amarus* proved effective against these negative changes in both serum and liver lipids, reducing heart and atherogenic risk indices. This further confirms the antimalarial activity of the plant. The roles of alkaloids and flavonoids in reversing dyslipidaemia in animal models have previously been reported [34]. Bhavsar et al [35] also reported that saponins ameliorate dyslipidaemia and related metabolic syndromes by promoting increased gene expression responsible for biosynthesis of glucose transporter-4, adipsin and peroxisome proliferator-activated receptor gamma, while simultaneously decreasing gene expression of fatty acid binding protein 4 and glucose-6-phosphatase.

Changes in plasma lipids concentrations have been reported during acute malaria in humans [2]. Biochemical studies on blood stage *Plasmodium* have observed that, during the blood stage of the *Plasmodium* parasite growth, the parasite demands an enormous amount of lipids [1]. Therefore, parasites scavenge free fatty acids from serum and other sources such as HDL [36]. They have also been reported to intercept cholesterol derived from LDL and the mevalonate pathway to aid sterol needs in liver stages [37]. This scavenging ability ensures survival in cholesterol deprived situations. Within the parasites, lipids can either be incorporated with or without modification by elongation or denaturation [36]. Lipids could also be incorporated into phospholipids, diacylglycerols, and TAG [38].

Host lipids have also been implicated in the formation of haemozoin, the malaria pigment [39]. Linoeic acid have been shown to be important in reducing ferrirrprotoporphyrin IX dimers in early stages of haemozoin formation [40]. Increased serum lipids can also be attributed to lipid peroxidation and hemolysis of infected and non-infected erythrocytes in hosts [41]. Since level of hemolysis is positively correlated with severity of infection [29], then, lipid concentration following malarial infection is positively correlated with severity. Also, hepatocytes profiling showed that *Plasmodium* infection induces an up regulation of sterol synthesis and lipid metabolism coding genes are up regulated in host cells [42]. These could account for changes in lipid content in malarial infection..

Liver, the major site of lipoprotein metabolism and cholesterol synthesis in humans, is also the major site for *Plasmodium* infection [39]. In malaria infection, major changes to the liver such as enlargement and congestion due to blockage of sinusoids and centrilobular veins is common. Swollen parenchyma and Kupffer cells has also been reported [43]. Hepatocellular damage associated with severe *Plasmodium* infections compromises the process of lipid homeostasis and metabolism [39]. This leads to modifications in plasma lipids and deformity of synthesized lipoprotein particles leading to lipolysis [44]. Increased lipolysis results in increased VLDL synthesis and hence, increased metabolism of LDL [45]. Therefore, increased parasitaemia subsequently induces an increase in LDL concentrations, leading to incorporation of cholesterol particles. Oluba et al [44]. hypothesized that increased LDL could be due to reduced breakdown of LDL as a result of damaged hepatocytes or decreased uptake by infected erythrocytes due to increased parasitaemia.

Lipids are important components in cardiovascular risk prediction and therapy in primary and secondary prevention of heart diseases [3]. Individuals with a history of malaria have a significantly increased risk of incident heart failure [5]. The alteration in serum lipid profile; increased TC, TAG, and LDL-C; and reduction in HDL-C are key factors in cardiovascular disease progression [46]. TAG level has been reported to be linked to risk of cardiovascular event, myocardial infarction, coronary heart disease and death in adult irrespective of the other components of lipid profile [47]. Hence, confirmation of elevated TAG alone is a good predictor of cardiovascular disease. High levels of LDL-C are mainly responsible for hypercholesterolemia. HDL-C is known to have antiatherogenic properties, while LDL-C constitute the primary carriers of plasma cholesterol which build up in the walls of the arteries, thereby causing atherosclerosis. Patients with arterial disease can have elevated LDL [48]. Therefore, the high levels of LDL-C and low levels of HDL-C in negative control mice from our study, confirms that malarial infection induces an increased risk of cardiovascular failure. Clinical investigations have revealed that individuals that exhibit high AIP run high risk of developing atherosclerosis and coronary heart disease [20]. Chikezie et al [49] shows a strong positive correlation between AIP and LDL-C concentrations and confirms that elevated levels of LDL-C contributed to antherogenecity. This is consistent with our study.
Studies have shown that extracts of *P. amarus* confer cardiovascular protection. Olorunnisola et al [50] showed that ethanol extracts of *P. amarus* displayed considerable cardio protection against high salt-diet that may lead to cardiovascular disease. Its hypolipidaemic activity has also been reported [51]. Nwankpa *et al* [52] reported the plant's ability to reduce hyperlipidaemia and lipid peroxidation in *Salmonella* *typhi* infection in mice. Increased atherogenic risk index in normoglycaemic mice is significantly reduced in mice treated with aqueous extracts of *P. amarus*, by lowering cholesterol and LDL-C index [53]. Yao *et al* [54] reported that aqueous extracts of *P. amarus* reduced acetate (DOCA)-salt-induced hypertension in rat model and also improved cardiac structure and endothelial function. They attributed this to its antioxidant properties.

**CONCLUSIONS**

The roots of *Phyllanthus amarus* harvested from Abraka, Delta State, Nigeria, is rich in both phytochemicals and nutrients, with demonstrated significance in antimalarial activity which reduced the risk of cardiovascular diseases, heightened by malarial infection in mice. This plants’ part is, therefore, a good source for antimalarial drug screening and should be explored.

**Acknowledgments**

We appreciate the technical assistance of Mr. Cyril Dunkwu, Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria and the Director, Affamefune, Biomedical Research and Consultant, Abraka, Delta State, Nigeria.

**Conflict of Interest**

No conflict of interest associated with this work

**Author contributions**

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Onyesom I. conceived and designed the study. Opajobi A.O., Uzuegbu U.E. and Toloyai P.Y. conducted the laboratory research experiment. Elu C.O. and Igwenyi I. analysed the data and wrote the manuscript. The manuscript was read by all authors and approved by submission.

**REFERENCES**


DOI: https://doi.org/10.15379/ijmst.v10i4.2268

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.