Preliminary Evaluation of the Physicochemical and Antiplasmodial Properties of Syrup Formulations Containing the Aqueous Root Extract of Nauclea latifolia (Rubiaceae)

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INTRODUCTION

Malaria is a major life-threatening disease with high rates of prevalence and mortality. There are about 200 - 250 million cases reported annually resulting in about 1 - 2 million deaths, 90% of which occur in Africa with Nigeria accounting for a quarter of all malaria cases in Africa [1]. Malaria is holoendemic in Nigeria; it is the most common cause of hospital visits in all age groups but particularly devastating to children under 5 years [2]. Early diagnosis and treatment with recommended therapies reduce the risks of complication, rate of transmission and death. However, resistance to available antimalarial agents has become a recurring problem resulting to various challenges in the management of malaria especially in areas where multi drug resistance is prevalent. This has consequently heightened the interest in the search for novel alternative treatments for malaria and especially the development of herbal medicines [3].

Herbal plants have been in use for the treatment of malaria for many years and are still being used in healthcare worldwide. About 80% of the population in developing countries depend on herbal medicines for their basic health needs [4] because they are purported to be as effective as conventional medicines while being cheap and relatively safe.

Nauclea latifolia; family; Rubiaceae, is one of those medicinal plants used traditionally in the treatment of malaria [5]. It is a deciduous flowering plant that is readily available in West Africa; its various parts have diverse applications locally [6]. The antimalarial beliefs of Nauclea latifolia has been investigated and reported to be effective in its claim by many researchers. The crude root extract of Nauclea latifolia has been observed to cure Plasmodium berghei infected rats [7] and mice. Its antioxidant property has been credited.

Keywords: Nauclea latifolia, dried root extract, syrup, honey, simple syrup, physicochemical evaluation.

Abstract

Malaria is a life-threatening disease with high mortality and prevalence especially in children under the age of 5. Herbal remedies like Nauclea latifolia have been used and found to be effective in the treatment of malaria. However, presentations like decoctions, liquid extracts are not suitable particularly for children. Therefore, in this study, syrup formulations of the dried aqueous root extract of Nauclea latifolia (AREL) were developed using honey, simple syrup BP and water as vehicle in the presence and absence of a sweetener, thickener and preservatives. Organoleptic and physicochemical properties such as odor, color, taste, homogeneity, pH and density were evaluated. Stability of the syrups at room temperature, in the refrigerator and at accelerated temperature was also evaluated. In vivo anti-plasmodial activity of optimized syrup formulation was investigated according to standardized methods. Results show the syrups were brown to amber in color, homogenous and had sweet to bitter taste. The pH of the syrups were between 4.45 and 5.78, flow rate was between 0.00 and 2.17 g/sec. Density was between 1.24 and 1.41 g/mL; syrups containing honey were more free-flowing. In vivo studies show that the optimized syrup formulation possess ability to inhibit parasitemia. No observable changes were noticed after the period of storage. This study shows that the development of the dried aqueous root extract of Nauclea latifolia into syrups as a standardized dosage form capable of inhibiting parasitemia could be explored.
Materials and Methods

**Materials**

Dried aqueous root extract of *Nauclea latifolia* obtained from the NIPRD laboratory, methyl paraben and propyl paraben (Tianjin Zhongxin Chemtech Co., Ltd, China), sugar and honey sourced from the National Institute for Pharmaceutical Research and Development, Laboratory, Nigeria.

**Methods**

Collection, Identification and Preparation of the dried aqueous root extract of *Nauclea latifolia*

The roots of the plant *Nauclea latifolia* were obtained from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Plant identification was done by the institute’s taxonomist, Muazzam Ibrahim and a specimen stored in the Institute’s herbarium with voucher specimen reference No 4251. The root bark was washed and dried in a drying cabinet and pulverized using a mechanical grinder. The powdered material was extracted using distilled water by hot maceration for 24 h, then filtered through a muslin cloth and freeze-dried using LYOVAC, GT2 (Germany). The freeze-dried extract was then stored in an air tight container and stored in a desiccator.

Preparation of the herbal syrups

Honey, simple syrup and distilled water were used as vehicle for the preparation of the herbal formulation according to the formula in Table-1 below. Appropriately weighed quantities of AREL were placed in a mortar and where required, the preservatives (methyl paraben and propyl paraben) and sweetener were mixed with the extract. A portion of the vehicle was added to the content of the mortar and triturated together to make a smooth paste. The remaining volume of vehicle was incorporated into the mortar and triturated until a homogenous mixture was obtained which was transferred into a bottle, labelled appropriately and stored until further use.

**Materials and Methods**

Table-1: Composition for the preparation of syrups containing AREL

<table>
<thead>
<tr>
<th>Ingredients/ Batch</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AREL (g)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Methyl paraben (g)</td>
<td>-</td>
<td>0.06</td>
<td>0.06</td>
<td>-</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Propyl paraben (g)</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Sweetener (g)</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Honey (mL)</td>
<td>60</td>
<td>45</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Syrup (mL)</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>45</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>Water (mL) to</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>-</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Key: AREL = Aqueous root extract of Nauclea latifolia; H1 and S1 = preparations containing only AREL in honey and AREL in simple syrup respectively; H2 and S2 = preparations containing AREL, preservatives and honey in water and simple syrup respectively; H3 and S3 = preparations containing AREL, preservatives, sweetener, honey in water and simple syrup respectively.
Physicochemical evaluation of syrup formulations containing AREL

Physical evaluation
The color, odor and taste of the syrups were evaluated.

Chemical evaluation
Determination of specific gravity at room temperature
The pycnometer was used to determine specific gravity (g/mL) of the syrups. The weight of the empty pycnometer and its weight when filled with water were recorded as \( W_1 \) and \( W_2 \) respectively. The weight of the syrup in the pycnometer and the weight of the pycnometer filled with equal volume of water were recorded as \( W_3 \) and \( W_4 \). Specific gravity was computed using the formula below:

\[
Specific \, gravity = \frac{W_3 - W_1}{W_4 - W_1} \quad ........ (1)
\]

Determination of density of syrup
The method of Tewari and Kumar [27], was adopted; the empty bottle of known volume was weighed (\( W_v \)) and then filled with syrup and weighed again (\( W_s \)). The difference between the weight of the empty bottle and the bottle containing the syrup was recorded (\( W_x \)) and density was calculated using the given formula:

\[
Density = \frac{W_x}{W_v} \quad ................. \quad ........... (2)
\]

Determination of pH
The pH of the syrups was determined using the digital pH meter (Denver pH meter). Three determinations were recorded and the average calculated.

Determination of flow rate
This was determined using the funnel method.

Evaluation of Syrup stability
The prepared syrups were stored at room temperature, in the refrigerator and at accelerated temperature (40 °C) for 30 days and assessed visually for presence/absence of growth or crystals.

Animals and ethics statement
Swiss Albino mice (24 – 28 g) of either sex were used for the study. These were obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. The animals were housed in plastic cages and maintained under ambient laboratory conditions. They were fed on standard rodent diet with free access to tap water. The study was carried out following approval obtained from the Institutional Animal Care and Ethics committee (NIPRD/05.03.05-6).

In vivo anti-plasmodial activity
This was determined by an established method [17]. The curative test was carried out in mice infected with chloroquine-sensitive Plasmodium berghei (NK-65). Donor mouse previously infected with Plasmodium berghei was sacrificed by diethylether inhalation. The thoracic region was opened and blood was collected by cardiac puncture into heparinized tube. Normal saline was used to dilute the blood to achieve a concentration of 1 x 10^7 parasitized red blood cells which was given intraperitoneally to each mouse on Day 0. After 72 h (Day 3), blood obtained from the tail of the mice was used to prepare thin blood films which was used to determine the level of parasitemia.

The mice were subsequently randomized into 5 groups of 6 mice each; mice in group 1 which served as negative control received SV; the vehicle of the formulation (10 mL/kg/day), groups 2 and 3 were administered 200 and 400 mg/kg/day of formulation S3. Group 4 which is the positive control, was treated with chloroquine (5 mg/kg/day) while group 5 was the untreated, uninfected group.

Treatment was administered once daily in a single dose for five (5) days i.e. Day 3 - Day 7. Thin blood smears were prepared at 2-day intervals then 24 h after the last treatment, blood was obtained from the tail of the mice for hematological analysis carried out using Wincom YNH7021-Diff hematology analyzer. The slides were Geimsa stained and the number of parasitized red blood cells and total number of cells were counted in randomly selected fields (n=10). Mortality was monitored over a 30-day period; percentage parasitemia, percentage of suppression and mean survival time was calculated using the formulae below:

\[
Parasitemia \, (\%) = \frac{\text{total number of parasitized RBCs}}{\text{total number of RBCs}} \times 100 \quad ........ .... \quad ........ (3)
\]

\[
Suppression \, (\%) = \frac{\text{parasitemia in negative control} - \text{parasitemia in treated group}}{\text{parasitemia in negative control}} \times 100 \quad ........ (4)
\]

\[
\text{Mean survival time} = \frac{\text{total number of days of survival of all mice in the group}}{\text{total number of mice in that group}} \quad ........ .... \quad ........ (5)
\]
STATISTICAL ANALYSIS
Values are presented as Mean ± SEM and analyzed using Graph Pad Prism V 6.01. Comparison were made between control and treatment group using One-way Analysis of variance (ANOVA). Followed by Dunnet’s multiple comparison test. Level of significance was set at p<0.05.

RESULTS AND DISCUSSION
Physical properties of syrups containing AREL
The physical properties of the formulated syrups are presented in Table (2). All the syrups were brown to amber in color, they were found to be odourless except those prepared with honey (H1, H2 and H3) which had a characteristic odor. Syrups prepared with honey (H1, H2, H3) were sweet to taste while those prepared with syrup (S1, S2 and S3) even though contained a sweetener were bitter. This is attributed to the unpleasant taste of the extract (AREL) due to the presence of alkaloids which is known to be bitter-tasting [18, 19]. Honey was found to mask the bitter taste of the extract, resulting in the sweet-tasting syrups.

The pH of the syrups were observed to be between 4.45 and 5.78. Syrups prepared with honey as the vehicle (H1) and those containing honey with water as vehicle (H2 and H3) were found to have similar pH with honey alone (4.42). On the other hand, the pH of formulations S1, S2 and S3 prepared with simple syrup as vehicle and its combination with water were 5.50, 5.63, 5.78 respectively which was lower than the pH of simple syrup alone (6.63). Although the presence of AREL is seen to lower the pH of the preparations, the influence was not remarkable.

Density of the syrups was observed to be between 1.24 and 1.41 mg/mL. Preparations containing honey as vehicle (H1) and simple syrup as vehicle (S1) were the densest although they were also similar (1.41 and 1.40 respectively). This was followed by those prepared with the combination of simple syrup and water (H2 and H3) which also had similar densities (1.31) while S2 and S3 which also had similar densities (1.24 and 1.28 respectively) were the least dense.

Flow rate measures how quickly fluid would move from one point to the other or through an orifice. The time taken for a viscous or dense fluid to flow out or through an opening would be longer than that of a less viscous fluid. This suggests that formulations S2 and S3 would be more easily dispensed from their final container than the other syrup formulations and this is important to ensure patient compliance and product efficacy. Table-2 shows that syrups prepared with honey and its combination with water had very poor flow which corresponds to the density of the preparations.

Table-2: Organoleptic and Physicochemical properties of formulated syrups containing AREL

<table>
<thead>
<tr>
<th>Parameters/ Batch</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
<td>Amber</td>
<td>Amber</td>
<td>Amber</td>
</tr>
<tr>
<td>Odor</td>
<td>Honey</td>
<td>Honey</td>
<td>Honey</td>
<td>Odorless</td>
<td>Odorless</td>
<td>Odorless</td>
</tr>
<tr>
<td>Taste</td>
<td>Sweet</td>
<td>Sweet</td>
<td>Sweet</td>
<td>Bitter</td>
<td>Bitter</td>
<td>Slightly bitter</td>
</tr>
<tr>
<td>pH</td>
<td>4.48</td>
<td>4.45</td>
<td>4.63</td>
<td>5.50</td>
<td>5.63</td>
<td>5.78</td>
</tr>
<tr>
<td>Flow rate (g/sec)</td>
<td>0.00 ± 2.00</td>
<td>0.25 ± 2.00</td>
<td>0.01 ± 2.52</td>
<td>0.45 ± 1.00</td>
<td>1.25 ± 1.00</td>
<td>2.17 ± 0.58</td>
</tr>
<tr>
<td>Density (g/mL)</td>
<td>1.40 ± 1.00</td>
<td>1.31 ± 1.16</td>
<td>1.31 ± 1.51</td>
<td>1.41 ± 2.00</td>
<td>1.24 ± 1.00</td>
<td>1.28 ± 0.56</td>
</tr>
</tbody>
</table>

Table-3: Organoleptic evaluation of formulated syrups containing AREL after storage at different temperatures for 30 days

<table>
<thead>
<tr>
<th>Batches</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 °C</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Room Temperature</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>40 °C</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>
Stability studies of the prepared syrups as presented in Table (3) shows that all the syrups retained their physical properties (colour, odour, presence/absence of growth) under the different storage conditions. Honey used in formulations H1, H2 and H3 possesses broad spectrum antimicrobial property [20] and maybe responsible for preserving the syrups under storage. Although simple syrup on the other hand is known to be bacteriostatic, the presence of preservatives (methyl paraben and propyl paraben) in formulations S1, S2 and S3 could account for the stability of the syrups observed.

Based on the pH, better flow rate and density of the formulated syrups, formulation S3 containing AREL prepared with combination of simple syrup and water and a sweetener was selected for the in vivo study.

A decrease in the count of parasitized erythrocytes is widely used as an indicator of antiplasmodial activity of a test agent. The reduction in percentage parasitemia observed in this study is an indication of the plasmodial inhibitory potential of the pharmacologically active components of the preparations. The formulated syrup (S3) exhibited antiplasmodial activity indicating that formulation of the extract did not affect the actions of the active constituents. Although, chloroquine exhibited the highest parasite suppression (74.32 %), the results also show the propensity of the formulated syrup to reduce parasitemia by 28.44 and 42.51 % at 200 and 400 mg/kg respectively. This suggests its potential as an anti-malarial formula.

The antiplasmodial activity of syrup formulation (S3) was found to be dose-dependent evident from the parasite clearance as shown in Table (4) with significant reduction (P<0.05) at 400 mg/kg. Reduction in parasitemia was also observed with the group treated with chloroquine at 5 mg/kg.

### Table 4: Anti-plasmodial effect of syrups containing AREL and survival time in mice infected with *Plasmodium berghei*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean % parasitemia (D7)</th>
<th>% Suppression</th>
<th>Mean survival time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SV</td>
<td>3.27 ± 0.83</td>
<td>-</td>
<td>8.67 ± 0.67</td>
</tr>
<tr>
<td>S3 200 mg/kg</td>
<td>2.34 ± 0.85*</td>
<td>28.44</td>
<td>10.00 ± 0.55</td>
</tr>
<tr>
<td>S3 400 mg/kg</td>
<td>1.88 ± 0.12*</td>
<td>42.51</td>
<td>11.17 ± 1.64</td>
</tr>
<tr>
<td>Chloroquine 5 mg/kg</td>
<td>0.84 ± 0.32*</td>
<td>74.32</td>
<td>19.17 ± 3.27*</td>
</tr>
</tbody>
</table>

Key: D7 = Day 7; Results are expressed as Mean ± SEM n=6; One-way ANOVA, *P<0.05, p<0.01, p<0.001, P<0.0001 compared treatment to negative control

**In vivo anti-plasmodial activity**

The antiplasmodial activity of the formulated syrup was determined using the curative in vivo antiplasmodial assay. There is a rise in the use of plant extracts for the treatment of diseases in man with these extracts being formulated into suitable oral dosage forms that have shown significant pharmacological actions [21, 22]. Extracts obtained from the plant have been reported to possess antimalarial properties [23]. Administration of the formulated syrup to mice with established infection showed a decrease in the level of parasitemia after five days of treatment (D7). The antiplasmodial action of syrup formulation (S3) was found to be significantly reduced (P<0.05) at 400 mg/kg. Reduction in parasitemia was also observed with the group treated with chloroquine at 5 mg/kg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Red Blood Cells (x10/L)</th>
<th>Hemoglobin (g/L)</th>
<th>Hematocrit (%)</th>
<th>Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SV</td>
<td>3.73 ± 0.42</td>
<td>162.33 ± 8.65</td>
<td>17.65 ± 2.06</td>
<td>126.30 ± 34.31</td>
</tr>
<tr>
<td>S3 200 mg/kg</td>
<td>4.45 ± 0.63</td>
<td>170.60 ± 9.35</td>
<td>20.57 ± 2.86</td>
<td>249.2 ± 13.46</td>
</tr>
<tr>
<td>S3 400 mg/kg</td>
<td>5.26 ± 0.78</td>
<td>221.80 ± 8.49</td>
<td>30.73 ± 2.34</td>
<td>227.0 ± 41.42</td>
</tr>
<tr>
<td>Chloroquine 5 mg/kg</td>
<td>6.75 ± 0.46*</td>
<td>222.33 ± 10.64*</td>
<td>30.87 ± 2.30*</td>
<td>411.3 ± 38.65*</td>
</tr>
<tr>
<td>Sham</td>
<td>16.52 ± 2.69*</td>
<td>376.60 ± 18.67*</td>
<td>58.66 ± 2.60*</td>
<td>1007 ± 30.81*</td>
</tr>
</tbody>
</table>

Key: D7 = Day 7; Results are expressed as Mean ± SEM n=6; One-way ANOVA, *P<0.05, p<0.01, p<0.001, P<0.0001 compared treatment to negative control

The presence of alkaloids, saponins, flavonoids, cardiac glycosides and tannins in *Nauclea latifolia*, may be responsible for its antimalarial activity [9] and this is observed to be exhibited in the syrup formulation. The mean survival time of the treated mice increased with dose but was shorter than the time observed in mice treated with the standard (chloroquine).

Malaria related anemia occurs in infections with the *Plasmodium* parasite often causing alterations in blood cell parameters. Hematological analysis carried out on mice with established infections show decreased counts of blood cells that include red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT) and platelet (Plt) count. Low blood cell count is associated with high parasite density caused by increased hemolysis and decreased rate of erythrocyte production [25].
Administration of the syrup (S3), showed increasing levels of blood parameters as displayed in Table (5) which were comparable with that of chloroquine. This effect may be attributed to decreasing parasite density as the treatment was administered and also hematoprotective property of *Nauclea latifolia* which has earlier been reported [26].

**CONCLUSION**

This study shows that the dried aqueous root extract of *Nauclea latifolia* can be developed into syrup formulations with acceptable physicochemical properties using the combination of simple syrup and water as the most suitable vehicle. *In vivo* studies show that formulation of the extract into syrups did not alter its parasite-inhibitory properties.

**Conflict of Interest:** The authors declare no conflict of interest.

**ACKNOWLEDGEMENT**

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