In *vivo* antiplasmodial activities of four Nigerian plants used singly and in polyherbal combination against *Plasmodium berghei* infection

Orabueze Celestina I*, Adesegun Sunday A, Ota Duncan A & Coker Herbert A

Department of Pharmacognosy University of Lagos, PMB 12003, Surulere, Lagos, Nigeria;
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, PMB 12003, Surulere, Lagos, Nigeria

E-mail: iorabueze@unilag.edu.ng

Received 03 October 2017, revised 14 August 2018

Methanolic extracts from 4 medicinal plants representing 4 families, used traditionally for malaria treatment in South east Nigeria were screened for their in *vivo* antimalarial activity in mice against a chloroquine (CQ)-sensitive *Plasmodium berghei* NK65, alone and in combination as polyherbal remedy. The methanolic extracts of individual plants in single and in combination (100-400 mg kg\(^{-1}\)) were administered orally to *P. berghei*-infected mice in both early and established models of antiplasmodial studies. Survival time was determined. When used alone, extracts from the 4 plants, *Fadogia cienkowskii* (FC), *Lophira lanceolata* (LL), *Vernonia conferta* (VC) and *Protea madiensis* (PM) had statistically significant parasitaemia suppression (62.06 – 93.44 %) and curative (48.93 – 72.47 %) effects. Lower doses of the 4 individual plants constituted FLVP at a combination ratio of 1: 1: 1: 1. Polyherbal formulation (FLVP) gave statistically significant suppression and curative which ranged from 45.5 – 85.1 % and 45.5 – 74.0 %, respectively. A more general improved antimalarial recovery effect, controlled weight lost and enhanced survival rate of the test mice compared to the individual plant therapeutic effect was observed. The standard drug, CQ gave stronger curative effect 100 % parasitaemia clearance. Our study findings suggest that the 4 plants used both as monotherapy and combined polyherbal remedy showed antiplasmodial in *vivo* activities and FLVP showed a more stable recovery status. FLVP is safe up to tested dose of 4000 mg kg\(^{-1}\). Further studies using varying fixed ratios for FLVP could result in better and improved antimalarial formulation.

**Keywords**: Antiplasmodial, Curative activity, Suppressive activity, Polyherbal formulation, Parasitaemia, Synergistic

**IPC int. Cl.**: A61K 36/00, A01D 20/46, A61K 39/00, A61K 31/47, A61K 31/4706, A61K 31/357

In Nigeria, malaria is one of the most prevalent infections and constitutes one of the main causes of death particular in children under the age of 5 yrs, pregnant women and immunity challenged adults\(^1,2,3\). Malaria is associated with many complications which include severe anemia, cerebral malaria, respiratory distress syndrome, and low birth weight, which contribute to final cause of death\(^4\). World Health Organization (WHO) records showed that about an estimated 303 000 under fives globally, including 292 000 in the African succumbed to malarial associated death in the year 2015\(^5\). Efforts to progressively reduce the high malaria mortality and morbidity rates is challenged by the ease at which the parasite, particularly *Plasmodium falciparum* develop resistance to available and inexpensive conventional drugs and vectors to insecticides\(^3,6\). Preventive effect of vaccine would have been a better approach towards keeping infection and re-infection of malaria under control but that may be in the near future since effective vaccine is still not available. At present the clinical strategy for malaria therapy concentrates in use of combination therapy as advised by WHO, while focusing on research for new and more effective drugs due to the continuous development of resistance by the causative agent to available drugs.

Medicinal plants consist of many bioactive phytoconstituents, exhibiting different pharmacological activities, mechanisms of actions and biochemical path ways\(^7,8\). The health benefit or risk may increase when a combination of medicinal plants is presented as poly herbal formulation. Fixed dose formulations or drug combination is currently preferred over a single drug in many multidrug resistant infections such as bacterial infections, malaria, tuberculosis and HIV\(^9\) because of increased efficacy. Development of parasite resistance to artemisinin and its combinations is being reported

\(^\ast\)Corresponding author
in some parts of the world and there is a need for more treatment options.10

A large number of medicinal plants have been reported as potential sources of antiplasmodial agents. These plants are used as single or as concoction of two or more plants. A polyherbal concoction of four (4) medicinal plants namely, Lophira lanceolata, Protea madiensis, Vernonia conferta and Fadogia cienkowskii is used in South east of Nigeria as antimalarial remedy. Lophira lanceolata Tiegh, is a medicinal plant found in West Africa, including Nigeria. It has been documented to exhibit antimalarial potentials. Other reported uses of the plant include pain management, intestinal troubles, fever, respiratory tract infections, dysentery, antidiarrheal and spasmyloytic properties.11,12 The stem bark of Protea madiensis Oliv, is used in the treatment of diarrhoea, amenorrhoea, malaria, fever, headache and dysentery.13,14 Vernonia conferta Benth., belongs to the family Asteraceae. In traditional medicine, the use of Vernonia species include, anti-microbial, anti-malarial, analgesics, stomachache, anti-inflammatory, cytotoxic, anti-oxidant, worm expellant, various gastro-intestinal disorders, jaundice, and hypolipidemic effects.15,16 Fadogia cienkowskii Schweinf (Rubiaceae), locally called ‘ufu-ewureje’ in Nigeria is used in relief of headache, malaria, general body debility, inflammation, diarrhoea and impotence.17,18

This study was carried out to evaluate the antimalarial effects of these plants used singly as monotherapy and when formulated as polyherbal product.

Materials and methods

Equipment/reagents

Giemsa stain (Sigma-Andrich chemical company, St. Louis, USA), Chloroquine phosphate, DMSO (Dimethyl sulphate), methanol and other chemicals and reagents used for this study were of analytical grade.

Plant samples collection and identification

Leaves of the plants were collected at Nsukka, Enugu state in February 2016. The plants were identified and authenticated by a taxonomist, Mr. Ozioko Fred, from the International center for ethnomedicine and drug development (INTERCEDD), Nsukka Enugu state. The herbarium specimens were deposited and the following voucher numbers assigned: Lophira lanceolata (INTERCEDD/005), Protea madiensis (INTERCEDD/010), Vernonia conferta (INTERCEDD/1609) and Fadogia cienkowskii (INTERCEDD/1607).

Experimental animals

Swiss albino mice weighing between 18-22 g were obtained from the College of medicine animal care center, Department of Physiology, College of Medicine of the University of Lagos. The animals were accommodated in a clean, well ventilated but grouped caging system. Suitable nutritional, uncontaminated food and water was provided at all times and their beddings frequently changed. The animals were allowed to acclimatize 7 days preceding the experiment. Dark and light cycles were maintained at 12 h each. The animals were used in accordance with the guideline and recommendation of the ethics committee on the use of animals for research of the University of Lagos ethical committee, Lagos, Nigeria.

Malaria parasite

The parasite, chloroquine sensitive Plasmodium berghei (NK-65 strain) was obtained from National Institute of Medical Research (NIMR) Yaba Lagos Nigeria. It was then maintained in University Animal House Laboratory by serial passage of the parasite in an uninfected mice.

Preparation of plant sample

The collected samples (4 plants) were cleaned and dried at room temperature for two weeks. The dry plant samples were ground into powder using pestle and mortar. Each of the powders obtained was used to prepare individual extract. Cold maceration technique was employed for extraction, using 3600 mL of 90% methanol and 400 g of each plant materials. The combination extract (FLVP) was prepared by cold maceration of the 4 plant raw materials at the ratio of 100: 100: 100: 100 mg. The filtrates were concentrated under vacuum using a rotary evaporator (Buchi Rota vapor, Germany) at 45 rpm and 40 °C to obtain the crude extracts. The dried extracts were stored in a refrigerator at 4 °C in air tight containers until used.

Phytochemical analysis of the crude extract

A preliminary phytochemical study of the 4 plant extracts were performed using standard procedures. Determination of acute toxicity of crude extract of the polyherbal combination, FLVP

The median lethal dose (LD50) was determined for estimating acute toxicity of the crude extract of the polyherbal formulation, FLVP in Swiss albino mice model using Lorke method (1983)19. This involved
oral administration of 3 different doses of the extract (1000, 2000 and 4000 mg kg\(^{-1}\)) to groups of 6 mice each. The animals were observed for manifestation of physical signs of distress and toxicity such as writhing, decreased motor activity, stressed respiration and death for a period of 7 days.

**Antimalarial activity of the extracts**

**Animal grouping**

Animals were grouped according to extract and dose level (Table 1).

**Chemo-suppression test**

A 4-day suppressive test on early infection was used for the evaluation as described by Peter *et al.*\(^{21}\). The animals were infected intraperitoneally with blood containing 1 x 10^7 parasitized red blood cells in 0.2 mL inoculum on first day (D\(_0\)). They were randomly divided into test groups of 5 mice each. Two hours post infection, all the test groups (Table 1) of the animals were orally administered with the extracts accordingly. Three different dose levels were used, 100, 200 and 400 mg kg\(^{-1}\) for each extract. Two (2) test groups of the animals received the reference drugs (chloroquine 10 mg kg\(^{-1}\) and artesunate 15 mg kg\(^{-1}\)) while the last group received the vehicle, 5 % DMSO at 0.2 mL. The treatment was repeated daily for 4 consecutive days (D\(_3\) - D\(_7\)).

**Curative model**

Evaluation of curative potential of the extracts were carried out as described by Iyiola *et al.*, 2011\(^{22}\). Eighty five mice were intraperitoneally inoculated with standard inoculums of 1x10^7 *P. berghei* NK-65 infected erythrocytes on the first day known as Day 0 (D\(_0\)). After 72 h (D\(_3\)) and confirmation of parasitaemia, the mice were randomly divided into groups (Table 1) of 5 mice each and dosed accordingly. Doses of 100, 200 and 400 mg kg\(^{-1}\) of each extract were given accordingly daily for 5 consecutive days (D\(_3\) - D\(_7\)), control groups received 10 mg kg\(^{-1}\) chloroquine and 0.2 mL of 5 % DMSO. Geimsa stained thin blood film was prepared from the tail of each animal daily for the 5 dosing days (D\(_3\) -D\(_7\)) and D\(_8\) (24 h post last drug administration) and examined microscopically to monitor parasitaemia level. The prepared slides were viewed under X 100 objective (oil immersion) light microscope. The number of deaths that occurred in each group over a period of 30 days (D\(_0\) - D\(_{29}\)) were noted and mean survival time (MST) for each group determined.

**Statistical analysis**

The analysis was carried out in n = 5 for all determinations and the results were expressed as mean ± SEM. The significance of difference between the controls and treated groups were determined using one-way analysis of variance (ANOVA). p < 0.05 was considered to be statistically significant.

**Results**

**Phytochemical test**

Results obtained from phytochemical screening of the extracts showed some secondary metabolites (Table 2).

**Acute toxicity test**

There was no mortality recorded in the mice upon oral administration even at 4 000 mg kg\(^{-1}\). This indicates that the experimental doses used are relatively safe.

### Table 1 — Animal grouping

<table>
<thead>
<tr>
<th>Extract/Drug</th>
<th>100 mg kg(^{-1})</th>
<th>200 mg kg(^{-1})</th>
<th>400 mg kg(^{-1})</th>
<th>Others mg kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.2 mL</td>
</tr>
<tr>
<td><em>Fadogia cienkowskii</em></td>
<td>FC</td>
<td>FC</td>
<td>FC</td>
<td>-</td>
</tr>
<tr>
<td><em>Lophira lanceolate</em></td>
<td>LL</td>
<td>LL</td>
<td>LL</td>
<td>-</td>
</tr>
<tr>
<td><em>Vernonia conferta</em></td>
<td>VC</td>
<td>VC</td>
<td>VC</td>
<td>-</td>
</tr>
<tr>
<td><em>Protea madiensis</em></td>
<td>PM</td>
<td>PM</td>
<td>PM</td>
<td>-</td>
</tr>
<tr>
<td>Polyherbal FLVP</td>
<td>FLVP</td>
<td>FLVP</td>
<td>FLVP</td>
<td>-</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Artesunate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
</tbody>
</table>
Antimalarial test

Chemosuppressive effects of methanolic extracts compared in malaria induced mice with P. berghei

All the extracts showed dose-dependent schizonticidal effects on the parasitaemia at the doses employed in the study (Table 3). These effects were statistically significant relative to the negative control (p < 0.05). The chemosuppressive potential of FLVP is lower than that of *Fadogia cienwoskii* (FC) and standard drugs (CQ and Art). *Fadogia cienwoskii* (FC) at 400 mg kg\(^{-1}\) has the highest chemosuppression of 93.44 %, the standard drugs CQ (10 mg kg\(^{-1}\)) and Art (15 mg kg\(^{-1}\)) caused 89.36 and 85.5 % suppression, respectively. Chemosuppressive activity of CQ was significantly less than the effect of 400 mg kg\(^{-1}\) of FC but stronger than those of the other 3 individual test extracts and extract of polyherbal combination (FLVP) of the 4 plants.

**Antiplasmodial effect of methanol extracts in singles and in polyherbal combination on established infection**

The extracts showed dose-dependent curative effect on the parasitaemia (Table 4). The percentage parasitaemia reductions observed in all the various extract treated groups were statistically significant relative to the negative control (p < 0.05). The standard drug (CQ) used gave a total clearance (100 %) of the parasites by the last day of the treatment, followed by the FLVP at 74.00 % and FC with 72.47 % parasitaemia clearance. The survival time of animals were improved particularly with CQ, FLVP and FC (Table 5).

**Discussion**

The use of polyherbal remedy is a commonly employed procedure by traditional healers in Africa.
There is little or no scientific documentation of therapeutic benefits, safety information of such combination or its advantage over monotherapy of the individual plants. The number of medicinal plants that constitutes a polyherbal mixture or combination could be dependent on patient presentation or traditional health giver discretion. Nefang, is a polyherbal antimalarial product in South West Region of Cameroon and consist of 6 different antimalarial herbs.

The present study evaluated anti-malarial properties of 4 Nigerian medicinal plants, *Lophira lanceolata* (LL), *Protea madiensis* (PM), *Vernonia conferta* (VC) and *Fadogia cienkowskii* (FC) individually and in combination (at fixed dose ratio of 1:1:1:1) to validate their ethnomedicinal claims. Comparative antimalarial studies of the various test extracts using two *in vivo* models showed that the individual plant extracts and co-combination to be potential antimalarial agents. The two models employed were, 4-day suppressive test for assaying candidates for early infection and Rane test which evaluates curative effects of drug agents on established infection.

The results indicated that all individual administration of the extracts of the 4 plants and the combination of the 4 plants significantly (*p < 0.05*) and dose-dependently inhibited growth of the parasite (chemosuppression). Maximum growth inhibition (93.44%) of *P. berghei* was observed at 400 mg kg$^{-1}$.
Table 5 — Mean survival time (MST) of each group on day 30 (D₀-D₃₀)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg kg⁻¹</th>
<th>Mean survival time (MST) of each group</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% DMSO</td>
<td>0.2 mL</td>
<td>7.63 ± 1.1</td>
</tr>
<tr>
<td>CQ</td>
<td>10</td>
<td>28.00 ± 0.0</td>
</tr>
<tr>
<td>FLVP</td>
<td>100</td>
<td>19.11 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>20.34 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>24.88 ± 1.4</td>
</tr>
<tr>
<td>FC</td>
<td>100</td>
<td>17.28 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>17.52 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>21.41 ± 1.02</td>
</tr>
<tr>
<td>LL</td>
<td>100</td>
<td>9.31 ± 1.36</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9.78 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>12.73 ± 2.24</td>
</tr>
<tr>
<td>VC</td>
<td>100</td>
<td>11.01 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>14.26 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>15.78 ± 0.98</td>
</tr>
<tr>
<td>PM</td>
<td>100</td>
<td>12.73 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>13.56 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>17.05 ± 2.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

of *Fadogia cienkowskii* (FC) which is significantly (p < 0.05) higher that the effect obtained by the 2 standard drugs used CQ (89.36 %) and Art (85.46 %). Chemosuppressive effects of FC and FLVP at 400 mg kg⁻¹ gave 93.44 and 86.52 %, respectively. Though, the result demonstrated that FLVP produced less effect compared to FC, however, a lesser amount of the individual medicinal plants were required to make up 400 mg kg⁻¹ of FLVP. Thus, reducing the amount of each individual medicinal plant in the formulation of FLVP could mean increase in safety, tolerability and patient acceptability. The observed control in weight loss in both FC and FLVP treated groups may be due to the ability of the test extracts to inhibit the growth of parasitaemia after parasite inoculation, thereby protecting the animals from the negative effects of malaria.

The co-administration of 4 medicinal plants (FLVP) in curative model significantly reduced the parasite load on daily bases and enhanced the survival time of the mice compared to those given the individual extracts in monotherapy.

Reduction of parasitaemia in single and combination (FLVP) treated groups showed an early onset (24 h post initiation of drugs) of antiplasmodial effect in established infection model. This indicates that no booster dose may be needed to initiate activity and suggest that the drugs can be used in emergency situation. However, PM, LL and VC, were sign of quick recrudescence was noticed 24 post last dose administration may need a supporting drug for prolonged and sustained antimalarial effect. The extract of FC showed an increased significant curative effect 24 h post last dose administration, suggesting a long acting potential. The curative activity of FLVP indicated an early onset and prolonged chemotherapeutic reduction of parasitaemia. Prolonged parasite reduction effect of FLVP may have been gotten from FC characteristics. This may explained the more prolonged MST recorded for FLVP than the individual monotherapies (Table 5). Prolonged MST of the test animals suggest that they lived longer compared with the negative control but were not fully protected by the administered drug/extract. The good curative potentials of FC and FLVP extracts suggest their ability to destroy trophozoites in the blood and/or prevent formation of gametocytes, thereby preventing re-infection of the mosquitoes and man.

Previous studies reported that individual medicinal plant components of polyherbal therapy could be acting as antimalarial, supporting agents and both. Ethnobotanical claims of the 4 plants that constituted FLVP indicated that some of the plants may be acting both as antimalarial and supporting agents. Antimicrobial, moderate antimalarial, antioxidants, antipyrexia, anemia and management of tiredness activities of *L. lanceolata* have been reported. These properties of LL could be useful in management of malaria fever complications and symptoms. Traditional and scientific documented uses of FC extract as analgesic, blood building, antipyrexia, relief of headache, management of tiredness activities of *L. lanceolata* have been reported. A species, *Fadogia agrestis* leaves has been reported to exhibit *in vitro* antiplasmodial activity against *Plasmodium falciparum*. Some species of *Fadogia* have been implicated in gousieke poisoning studies but toxicity has been linked to seasonal variation, few species, high moisture content and very large dose (one-third of body weight). The genus *Vernonia* has documented antimalarial and malarial symptom relief activities for some of its species. Documented exploitation of therapeutic potentials of most of the plants used in this study is limited and there is a need to explore more possible medical and otherwise usage.

Similar records of combined extracts giving good clearance and suppression have been reported by
several authors which include Kingsley et al., (2012), Paula et al., (2012) and Osei-Djarbeng et al., (2015)\textsuperscript{33,8,21}. No sign of toxicity or death was observed in oral acute toxicity evaluation of FLVP polyherbal combination given up to 4,000 mg kg\textsuperscript{-1}. No literature search indicated toxicity of any of the plants that constituted FLVP poly herbal combination\textsuperscript{13,38}.

Many bioactive plant secondary metabolites are responsible for the observed anti-malarial activity but the most important and diverse bio-potencies have been observed in alkaloids, quassinoids, flavonoids and sesquiterpene lactones\textsuperscript{34}. All the 4 plants that constituted FLVP presented alkaloid and flavonoid.

Our study appears to be the first scientific investigation report on antimalarial effects of \textit{Fadogia cienkowskii} and \textit{Protea madiensis}, to the best of the authors knowledge.

**Conclusion**

Although it is premature to conclude at this stage that this herbal combination can be used as effective antimalarial, the findings provide a foundation for further exploration of possible therapeutic benefit of herb-herb combinations for prevention and management of malarial infections. The results obtained in this study may not have indicated synergistic or remarkable additive effect but showed that the medicinal plants that constituted FLVP may have contributed diverse pharmacological actions which may have contributed towards a more stable and long acting healing effect. Thus, the end product may possibly slowed down the rate of development of resistance, protect against side effects and increased survival rate of the animal. This study therefore, offers a scientific basis for the traditional use of these herbs separately and in combination against malaria parasite. Further studies on the herbs, safety, pharmacokinetic properties and optimizing its composition for maximum efficacy is highly recommended towards getting a better end product.

**Conflict of Interests**

We declare that we have no conflict of interest.

**Acknowledgement**

The authors acknowledge and are grateful for the financial support of TETFUND in the form of research grant No: CRC/TETFUND/No. 2014/01.

The authors are indebted to Mr. Ozioko F. and Nwafor F. of International Centre for Ethnomedicine and Drug Development (Inter CEDD), Enugu state, Nigeria for their aid in collection and identification of the plants.

**References**


