



In vivo evaluation of antiplasmodial activity of hydroethanolic stem extract of *Baphia pubescens* in *Plasmodium berghei* infected albino mice

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ABSTRACT

Introduction: Malaria is a global concern especially with the emergence of multidrug resistant strains of *Plasmodium falciparum*. The need exist for newer and effective antimalarial agents. Hydroethanolic stem extract of *Baphia pubescens* has been used for the treatment of malaria in traditional medical settings. This study explores the antimalarial potential of this extract.

Methods: The lethal dose of hydroethanolic stem extracts of *B. pubescens* was determined. Phytochemical screening of the extract was done using standard methods. Experimental animals received graded doses of the extract once daily for 3 days. In vivo antiplasmodial activity was assessed by the Rane's curative test, using *P. berghei* infected mice.

Results: The extract demonstrated a dose-dependent curative activity at the tested doses (100, 200 and 400 mg/kg). Complete (100%) parasite clearance was achieved (on day 4 post-treatment) at 400 mg/kg dose, which is greater than the standard drug used (artemether-lumefantrine). The extract is considered safe since the lethal dose was higher than 5000 mg/kg.

Conclusion: This study demonstrated that hydroethanolic extracts of *B. pubescens* has promising antimalarial activity. This supports the local use of *B. pubescens* as an anti-malarial agent. Further studies need to be done to identify and characterize the active principles/substances in the extract.

Implication for health policy/practice/research/medical education:

This study demonstrates that the stem part of *Baphia pubescens* possesses antimalarial activity. This plant can be explored as a source of a potential lead antimalarial molecule. Secondly, it can be used for the formulation of a standardised antimalarial herbal formula.

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Introduction

There is an urgent need to increase efforts in antimalarial drug discovery in order to develop safe and affordable new drugs to counter further spread of the malaria parasite (1). The emergence of resistant strains necessitates the introduction of potent new drugs or drug combinations against malaria. Preferably, new drugs should have novel modes of action or be chemically different from the drugs in current use (2).

Medicinal plants, a validated source of new medicines, have been used in virtually all cultures at one time or the other (3). It remains a source of safe and effective drugs (3). As a rich source for new drug molecules, several im-

portant antimalarial medicines in use today were either obtained or derived from them (4). Quinine and artemisinin are natural products from *Cinchona* bark and *Artemisia annua*, respectively.

Baphia pubescens Hook.f (family Leguminosae) is a plant which has been reviewed (5). The plant, also known as *Baphia bancoensis* Aubrev, has been demonstrated to possess anti-inflammatory property. The leaf extract has been reported to possess antinociceptive (analgesic) activity, too (6).

Though, scientific information on the antiplasmodial activities of *B. pubescens* is scarce, ethnomedicinal use indicates the plant might possess antimalarial property.

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Furthermore, a number of antimalarial plants also possess anti-inflammatory property. Popular among them are *Azadiracta indica*, and *Artemisia annua* (7-10). In view of its ethnomedicinal use, its anti-inflammatory property, and the incidence of association of anti-inflammatory and antimalarial properties, we decided to screen the plant for antimalarial properties. Consequently, this work investigated the in vivo antiplasmodial potential of the stem extract of *B. pubescens*.

Methods

Plant material

Source

Plant parts (including stems) of *B. pubescens* were collected in December 2014 from Ogidi, Idemili North of Anambra State Nigeria, and were authenticated by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Nsukka, Enugu State, Nigeria. The voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Agulu Campus (Herbarium Number PCG-474/A/021).

Preparation of extracts and fractions

The stems were air-dried at room temperature over a 7-week period, after which they were crushed using a dry mechanical mill. The hydroalcoholic extraction solvent used consisted of water and 95% ethanol in the ratio of 30:70, respectively. A quantity (0.3 kg) of the crushed stem fiber was macerated in 1.5 L of the extraction solvent for 72 hours, then filtered (Whatman® no. 1), and the filtrate evaporated to a paste on a thermostatically controlled water bath at 40°C. The extractive value was determined using a volume (1 mL) of filtrate in an evaporating dish (11).

Acute toxicity study and phytochemical screening

Acute toxicity study was done according to a modified method employed by Diet Riech Lorke (12) using albino mice. This method has two phases. In phase 1, groups (n = 3) of animals received oral doses of either 10, 100 or 1000 mg/kg body weight of the extract. In the second phase, another different set of mice were randomized into 3 groups (n = 3) and each group received either 1600, 2900 or 5000 mg/kg of the extract. All animals were monitored for signs of toxicity and mortality. The oral median lethal dose was calculated using the formula: $LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$. Phytochemical screening tests for the extract were also carried out using standard procedures (11,13).

Animals and parasites

Swiss albino mice (15–21 g, 4–6 weeks old) of either sex used for the study were obtained from the animal house of the College of Medicine, University of Nigeria, Enugu, Nigeria. They were housed under standard environmental conditions of temperature (22–29°C) and 12 hours dark-light cycle, and allowed free access to drinking water and standard pellet diet. *P. berghei* (NK 65) was obtained from

National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The experimental protocols used in this study were approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka Nigeria.

Drug

Pure samples of artemether and lumefantrine, which served as the positive control drug, were a gracious gift from Paucop Pharmaceuticals, Awka, Nigeria.

Experimental design

Parasite inoculation

All animals were quarantined for 7 days prior to infection. Blood from mice infected with *P. berghei* was used to infect the animals used for the experiment. Standard inoculums of 1×10^7 *P. berghei* infected erythrocytes in 0.2 mL were prepared by diluting infected blood with 0.9% normal saline. Each mouse was inoculated by intra-peritoneal injection with a blood suspension (0.2 mL) containing 1×10^7 parasitized erythrocytes (14). The parasite was maintained by serial passage of blood from infected to non-infected mice on a weekly basis.

Effect of extract on curative test

The evaluation of the curative potential of the extract in Swiss albino mice was done using the methods described by Ryley and Peters (15). Thirty mice were used for the experiments. Infected animals were divided into 5 groups (n = 5) when the level of parasitemia was observed to be > 4%. The extract was tested at 3 dose levels (100, 200, and 400 mg/kg/b.w./day). Three control groups (n = 5) were used namely, normal (uninfected and untreated), positive (infected and treated with 8 mg/kg of a formulation containing 20 mg artemether and 120 mg lumefantrine), and negative (infected and treated with distilled water). All treatments lasted for 3 consecutive days. Blood samples were collected from the tip of the tails of the animals on day 4 and day 7 post-treatment.

Parasitemia monitoring

Parasitemia was monitored using a previously described method (16). Briefly, blood samples were collected from the tip of the tails of the animals on day 4. Thin, blood films were dried, and fixed (for 15 minutes) using methanol, and subsequently stained with 10% Giemsa for 25 minutes. Stained film was washed off using phosphate buffer, pH 7.2 and allowed to dry. The film was immersed in oil and viewed at x100 magnification. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random fields of the microscope (17).

Average percentage parasitemia was calculated using the formula:

$$\% \text{Parasitemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}} \times 100$$

Average percentage of chemosuppression was calculated

using the formula:

$$\% \text{Suppression} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in test group}}{\text{Parasitemia in negative control}} \times 100$$

Data analysis

The results were presented as the mean \pm SEM (standard error of mean) for each group of experiments. The test groups were compared with the negative control group using one-way analysis of variance (ANOVA). All data were analyzed at a 95% confidence interval. P-values less than 0.05 were considered statistically significant.

Results

Acute toxicity and phytochemical screening

All the animals survived at 5000 mg/kg per oral test dose. Physical and behavioral observation of the experimental mice revealed no visible signs of acute toxicity such as hair erection, weakness and reduction in their motor and feeding activities. Though, the result of the phytochemical screening showed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, protein and terpenoid. It failed to detect the presence of steroids. The residue after evaporation was 52 g, which is 17.3% yield.

Effect of extract on curative test

On day 4, the extract suppressed parasitemia by 66.00%, 82.69% and 94.23% at 100, 200 and 400 mg/kg doses, respectively (Table 1). Similarly, on day 7 the extract reduced the parasite load by 90%, 96.15% and 100% at 100, 200 and 400 mg/kg doses, respectively (Table 2). The result showed that 100 mg/kg afforded 66.00% and 90.00% suppression on day 4 and day 7, respectively. At

Table 1. Antiplasmodial effect of hydroethanolic extract of the stem of *B. pubescens* in *P. berghei*-infected mice on day 4

| Dose (mg/kg bw) | Parasite control | |
|----------------------------|-----------------------------|---------------|
| | % Parasitemia | % Suppression |
| NC dH ₂ O | 5.4 \pm 0.78 | 0.00 |
| 100 | 1.7 \pm 0.33 ^a | 66.00 |
| 200 | 0.9 \pm 0.33 ^a | 82.69 |
| 400 | 0.3 \pm 0.20 ^a | 94.23 |
| PC Artemether/lumefantrine | 0.6 \pm 0.29 ^a | 89.29 |

Abbreviations: NC, negative control; PC, positive control.

^a Statistically significant ($P < 0.01$).

Table 2. Antiplasmodial effect of hydroethanolic extract of the stem of *B. pubescens* in *P. berghei*-infected mice on day 7

| Dose (mg/kg bw) | Parasite control | |
|----------------------------|-----------------------------|---------------|
| | % Parasitemia | % Suppression |
| NC dH ₂ O | 7.6 \pm 0.29 | 0.00 |
| 100 | 0.5 \pm 0.15 ^a | 90.00 |
| 200 | 0.2 \pm 0.12 ^a | 96.15 |
| 400 | 0.0 \pm 0.00 ^a | 100.00 |
| PC Artemether/lumefantrine | 0.1 \pm 0.10 ^a | 98.21 |

Abbreviations: NC, negative control; PC, positive control.

^a Statistically significant ($P < 0.01$).

400 mg/kg, the values were 94.23% (day 4) and 100% (day 7). The positive control had 89.29% (day 4) and 98.21% (day 7).

Discussion

The result of our study shows that the hydroethanolic stem extract possesses dose-dependent antiplasmodial activity as evident from the parasite clearance test. The result of the curative test at 400 mg/kg is similar to standard drug, artemether-lumefantrine (8 mg/kg/day).

The pharmacological activities of medicinal plants are believed to arise from its constituent phytochemicals. A number of phytochemical groups may be responsible for the observed activity. Alkaloids are popular for their toxicity against cells of foreign organisms such as bacteria, viruses and protozoans (to which malaria parasites belong). Quinine is a popular antimalarial alkaloid in current clinical use. Saponins are another group associated with antiprotozoal activity (18,19). The mechanism of action by which saponins work might be through their detergent effect on cell membranes (20). Another mechanism of action could be elevation of red blood cell oxidation or by inhibiting protein synthesis in parasite (2). The LD₅₀ of the hydroethanolic stem extract suggest the plant is safe for human consumption.

Conclusion

The result of this study shows that the stem extract of *B. pubescens* possesses antimalarial activity. No adverse effect was noticed when the extract was orally administered, alluding that the extract is not acutely toxic when administered to mice through this route. This supports its use in ethnomedicine in the treatment of malaria. Furthermore, it demonstrates its potential as a source of an antimalarial drug molecule. However, the findings are only preliminary, therefore, confirmatory studies, followed by isolation and characterization of the active antimalarial compound(s) responsible for the observed curative activity are recommended.

Authors' contributions

CAA introduced the concept and designed the work. KOC did laboratory experiments and captured data. CAA, KOC and CPI did data analysis. SCA prepared the initial draft. CPI revised the draft and produced the final manuscript.

Conflict of interests

The authors of the manuscript declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) were completely observed by the authors.

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References

1. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. *Nat Rev Drug Discov.* 2004; 3:509-20.
2. Muregi FW, Ishih A, Miyase T, et al. Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. *J Ethnopharmacol.* 2007;111:190-5.
3. Ntie-Kang F, Onguéné PA, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malar J.* 2014;13:81.
4. Basco LK, Mitaku S, Skaltsounis AL, et al. In vitro activities of furoquinoline and acridone alkaloids against *Plasmodium falciparum*. *Antimicrob Agents Chemother.* 1994;1169-71.
5. Anowi CF, Onyekaba T, Utoh-Nedosa U, Obi PE. An investigation into the antipyretic activity of n-hexane extract of the leaves of *Baphia pubescens* Hook. F (family Leguminosae). *J Pharmacogn Phytochem.* 2014;3:127-131.
6. Anowi CF, Onyekaba TU, Obi PE, Ezea CC. Anti-Inflammatory, phytochemical and toxicological investigations of ethanol extract of the leaves of *Baphia pubescens* Hook.F (Family Leguminosae). *Asian J Biochem Pharm Res Issue.* 2014;2:246-54.
7. Patel MB, Kadakia VM, Mishra SH. Simultaneous estimation of andrographolide and wedelolactone in herbal formulations. *Indian J Pharm Sci.* 2008; 70:689-93.
8. Chukwujekwu JC, Van Staden J, Smith P. Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. *South African J Bot.* 2005;71:316-25.
9. Melillo De Magalhes P, Dupont I, et al. Anti-inflammatory effect and modulation of cytochrome P450 activities by *Artemisia annua* tea infusions in human intestinal Caco-2 cells. *Food Chem.* 2012;134:864-871.
10. Patel DK, Dhanabal SP. Development of bioanalytical parameters for standardization of *Azadiracta indica*. *Asian Pacific J Reprod.* 2013;2:132-5.
11. Trease G, Evans W. *A Textbook of Pharmacognosy.* 13th ed. London: Bailliere Tinnall Ltd; 1989.
12. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol.* 1983;54:275-87.
13. Richardson PM, Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 2nd ed. Brittonia: Springer; 1990. p. 115.
14. Moll K, Ljungström I, Perlmann H, Scherf A, Wahlgren M. *Methods in Malaria Research.* Manassas: University Boulevard; 2008.
15. Ryley J, Peters W. The antimalarial activity of some quinone esters. *Ann Trop Med Parasitol.* 1970;84:209-22.
16. Arrey Tarkang P, Okalebo FA, Ayong LS, Agbor GA, Guantai AN. Anti-malarial activity of a polyherbal product (Nefang) during early and established *Plasmodium* infection in rodent models. *Malar J.* 2014;13:456.
17. Toma A, Deyno S, Fikru A, Eyado A, Beale A. In vivo antiplasmodial and toxicological effect of crude ethanol extract of *Echinops kebericho* traditionally used in treatment of malaria in Ethiopia. *Malar J.* 2015;14:196.
18. Wallace RJ, Arthaud L, Newbold CJ. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. *Appl Environ Microbiol.* 1994;60:1762-7.
19. Newbold CJ, el Hassan SM, Wang J, Ortega ME, Wallace RJ. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. *Br J Nutr.* 1997;78:237-49.
20. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. *Br J Nutr.* 2002;88:587-605.