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ANTIMALARIAL ACTIVITY OF ETHANOLIC EXTRACT OF *BAUHINIA MONANDRA* IN *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT

Introduction: Malaria remains an infectious illness consistently associated with significant levels of sickness, death, and resistance to medications.

Aim: This study evaluated the antimalarial activity of the ethanolic leaf extract of *Bauhinia monandra* in *Plasmodium berghei-infected* mice.

Methods: Intraperitoneal inoculation of resistant ANKA clones of *P. berghei* was performed on 25 mice (5 per group), while 5 mice remained uninfected as a control (Group 6). Group 5 was infected and received distilled water only, and Group 4 was treated with artemisinin combination therapy (ACT). Groups 1, 2, and 3 received oral doses of *B. monandra* extract at 50, 100, and 150 mg/kg body weight for three days, respectively. Parasitaemia levels were determined using the microscopic method. The extract was assessed for its phytochemical components following established methods. Also, Fourier-transform infrared spectroscopy analysis of the plant was carried out using standard methods. The effect of the leaf extract on hematological parameters and liver enzymes was also evaluated using standard methods.

Results: Results exhibited a significant ($p<0.05$) dose-dependent reduction in parasitemia among mice treated with *B. monandra* throughout the treatment period. The findings indicated that alkaloids, flavonoids, saponins, tannins, cardiac glycosides, phenols, and steroids present in the plants were 1.36%, 5.15%, 4.18%, 1.10%, 3.30%, 3.11 mg/l, and 0.083%, respectively.

Conclusion: These findings underscore the potential of *B. monandra* as a promising antimalarial agent due to its substantial impact on parasitemia and the presence of essential phytochemicals.

Keywords: *Bauhinia monandra*, percentage reduction, *Plasmodium*, hematological parameters, Enzyme, and mice

1.0 INTRODUCTION

Malaria is an illness transmitted by mosquitoes, affecting both humans and other animals, and caused by single-celled organisms known as eukaryotic protists belonging to the *Plasmodium* genus[1]. It is an

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age-old ailment that has likely resulted in more pain and fatalities than any other contagious illness, primarily affecting young children under the age of 5, particularly in developing nations[2-4]. In 2021, approximately 619,000 individuals died due to malaria, while there were an estimated 247 million new cases of malaria reported in the same year[5]. The majority of malaria cases are attributed to *P. falciparum* and *P. vivax*, where *P. falciparum* specifically leads to significant illness and is responsible for the majority of deaths related to the disease.

However, *P. falciparum* has acquired resistance to nearly all available antimalarial medications, making artemisinin-based combination therapy (ACT) the primary treatment for uncomplicated *falciparum* malaria across almost all regions. The recent evidence of *falciparum* multidrug resistance to ACT in Western Cambodia is worrisome[7]. However, there is deep concern that resistance of the parasite to this treatment will soon become widespread, and complete resistance to this orthodox treatment may emerge.

In light of this circumstance, the current situation in malaria treatment demands an immediate need for alternative antimalarial medications. It has been demonstrated that plant extracts are quite helpful in treating a variety of ailments[8, 9]. Medicinal plant extracts contain numerous naturally occurring inhibitors and pharmacologically active chemicals and have tremendous benefits for the treatment of various diseases[10]. Despite the heavy reliance of approximately 80% of Africa's population on traditional medicine, particularly plant-based remedies, for addressing illnesses like malaria, the full potential of plants in this context remains significantly unexplored[11], and this has increased the search for an alternative malaria treatment.

B. monandra is a leguminous tree that is often found in Thailand and is a member of the Fabaceae family. It has been used locally to cure cancer, alcohol poisoning, fever, and allergies[12]. Moreover, other uses of these plants have also been elucidated[13]. However, there is no reported study on its effect in malaria. Hence, this study evaluated the antimalarial activity of the leaf extract of *B. monandra* on *P. berghei-infected* mice.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection and identification of the plant

The identification of the plant, which was collected from Ndunwamini Amagu, Abakaliki Local Government Area of Ebonyi State, Nigeria, was made by a plant taxonomist at the Applied Biology Herbarium Centre, Ebonyi State University, Abakaliki, Nigeria.

2.1.2 Animal

Thirty healthy, two-month-old male mice were acquired from the Animal House of Veterinary Medicine at the University of Nigeria, Nsukka (UNN), for the experimental study. These mice were housed in metal cages within a controlled environment at the Presco Campus of EBSU. They were allowed unrestricted access to food and water. The animals were acclimatized for one week, and the experiment strictly adhered to the guidelines outlined by the National Institutes of Health (NIH) for the ethical and appropriate use of animals in research, ensuring their welfare and ethical treatment throughout the study.

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2.1.3 Parasites

This study utilized the *P. berghei* strain (chloroquine-resistant), specifically the ANKA strain, which was acquired from the Department of Veterinary Medicine at UNN.

2.1.4 Drug

The standard drug, Lonart DS, manufactured by CIPLA LTD, India, was used for the study.

2.2 METHODS

2.2.1 Processing and extraction of plant material

After the plant was cleaned, separated, and dried away from direct sunlight, it was finely processed into a powder with an electric mill and sieved. The extraction of the plant extract was conducted using a Soxlet apparatus by adding 200 g of the plant powder to 600 mL of 70 % ethanol using a continuous extraction method involving repeated cycles of solvent boiling and condensation to efficiently extract compounds from the plant material using a rotary evaporator (RE301 USA), and the residue was stored in a freezer (HR 170T, Japan) at 4 ^{o}C.

2.2.2 Phytochemical screening

The extract was assessed for its phytochemical components following established methods outlined by Trease and Evans[14].

2.2.3 Functional group analysis

Functional groups present in the plant sample were determined using Fourier-transformed infrared spectroscopy following the method of Maobe and Nyarango[15].

2.2.4 Determination of parasite density and inoculum preparation

The parasitized group of mice received a standardized inoculum containing 10^6 of *P.berghei*, suspended in 0.2 ml of phosphate-buffered saline (Vet Ulay Industries, UK), which was sourced from a donor mouse.

2.2.5 Experimental design

In this research, 30 mice were randomly divided into six groups, each containing five mice. All groups, except the normal control, received an intraperitoneal injection of 10^6 parasitized erythrocytes. Oral treatment began on the third day after the infection and continued for three days.

Groups 1, 2, and 3: Mice in these groups were infected with *P. berghei* and received treatments of 50, 100, and 150 mg/kg body weight of the extract, respectively.

Group 4: Mice in this group were infected with *P. berghei* and treated with a combination of artemether (80 mg) and lumefantrine (480 mg).

Group 5: Mice in this group were infected with *P. berghei* but did not receive any treatment.

Group 6: Animals in this group were neither infected nor subjected to any treatment.

2.2.6 Determination of parasitemia

Blood samples were obtained from mice infected with *P. berghei* by drawing blood from their tail veins, and parasitemia was determined microscopically after being stained with 10 % Giemsa stain.

2.2.7 Statistical analysis

The data were expressed as means of percentages, and each data set was compared using a one-way analysis of variance using the Software Statistical Package for Social Sciences (SPSS).

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3.0 RESULTS

3.1 Effect of treatment on parasitemia

The results demonstrated that, on the first day post-treatment, groups treated with 50, 100, and 150 mg/kg of B. monandry ethanol leaf extract exhibited reductions in parasitemia by 20.098%, 35.257%, and 49.119%, respectively. In comparison, the standard drug showed a 32.301% reduction in parasitemia. However, by the second day, there were higher reductions observed at 38.921%, 67.621%, and 92.873% for the respective extract doses, while the standard drug exhibited an 83.044% reduction. Moving to day three, the 50 mg/kg, 100 mg/kg, and 150 mg/kg groups displayed reductions of 58.833%, 69.017%, and 96.510% in parasitemia, whereas the standard drug recorded an 84.006% reduction (as depicted in Figure 1).

Over time, there was a gradual decrease in the percentage of parasitemia in all treated groups, with complete parasite removal observed by the 6th day in the groups treated with 150 mg/kg of the extract and ACT. Moreover, throughout the treatment duration, the extract exhibited an increase in the reduction of parasitemia in a dose-dependent manner. Notably, the 150 mg/kg dose of the extract demonstrated a significantly higher reduction ($p < 0.05$) in parasitemia compared to the ACT-treated group consistently from the first to the third day.

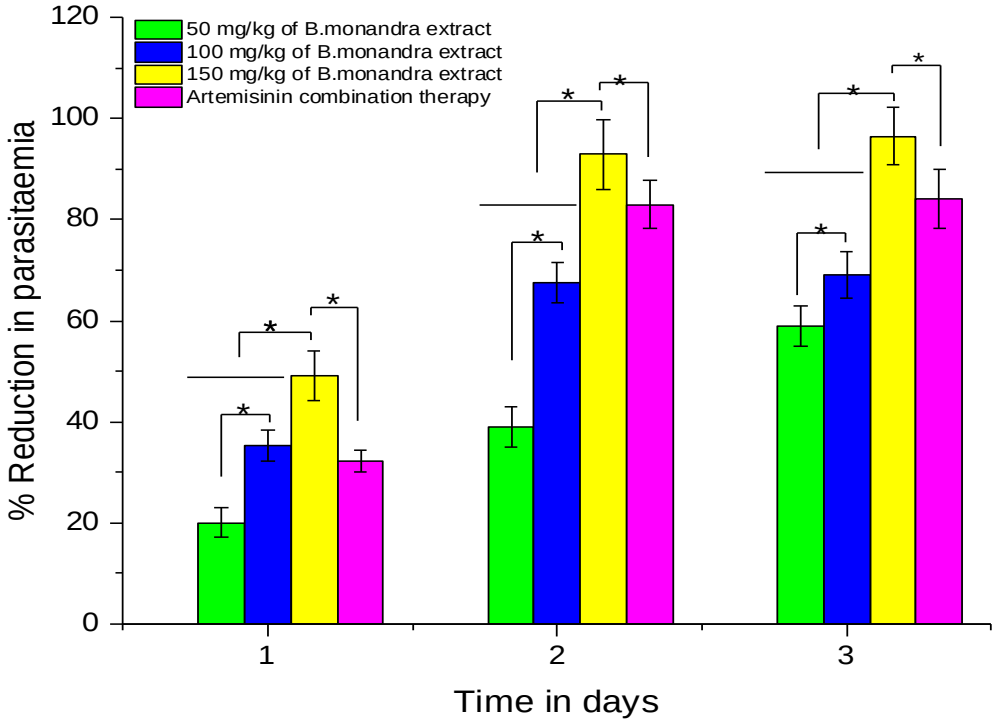

Figure 1: Effect of Treatment on Parasitemia Reduction
*** Significantly higher than ACT at $p < 0.05$**

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3.2 Phytochemical Studies

The phytochemical screening estimation of the percentage composition in *B. manandra* extract is presented in Table 1 below. As seen in Table 1, alkaloid, flavonoid, saponin, tannin, cardiac glycoside, phenol, and steroid content were recorded as 1.36%, 5.15%, 4.18%, 1.10%, 3.30%, 3.11 mg/l, and 0.083%, respectively. The anti-nutrients analyzed were phytate (0.014%) and oxalate (3.369 mg/kg).

Table 1: Phytochemical Constituents of the Ethanol Leaves Extract of *B. monandra*

S/N	Parameters	Concentration
1	Alkaloid	1.36%
2	Flavonoid	5.15%
3	Saponin	4.18%
4	Tannin	1.10%
5	Cardiac glycoside	3.30%
6	Phenol	3.11mg/l
7	Steroid	10.083%
8	Phytate	0.014%
9	Oxalate	3.369mg/kg

3.3 Fourier-transformed infrared spectroscopy (FTIR) of *B. monandra*

The functional groups contained in the active components were identified by FTIR analysis of the individual peak values in the infrared radiation range. The FTIR spectra are shown in Figure 2 below, while the interpreted spectra are recorded in Table 2. From Table 2, the peaks at 812.5119 cm^{-1} and 998.7845 cm^{-1} were assigned to the C-CI and CO stretch of chloro and ether compounds, respectively. The medium bands around 1260.429 cm^{-1}, 1688.547 cm^{-1}, and 3243.600 cm^{-1} were due to N-H stretching vibration of primary (10), secondary (20), and tertiary (3^0) amine compounds present in the leave samples. Absorbance around 1902.843 cm^{-1}, 2053.420 cm^{-1}, 2312.166 cm^{-1}, and 2412.247 cm^{-1} correspond to SCN, -CO, $R_2C{=}O$ and C≡N anti-symmetric stretching vibration of thiocyanate, carboxylic, carbonyl and nitrile compounds respectively. The peak heights of 2626.771 cm^{-1} and 2834.204 cm^{-1} were assigned to the C-H stretch of the methylene compound. The broad bands around 3024.741 cm^{-1}, 3121.274 cm^{-1}, 3491.580 cm^{-1}, 3719.331 cm^{-1}, and 3874.876 cm^{-1} correspond to OH stretching vibration of 1^0 and 3^0 alcohol found within the ethanolic extract of *B. monandra* leaves.

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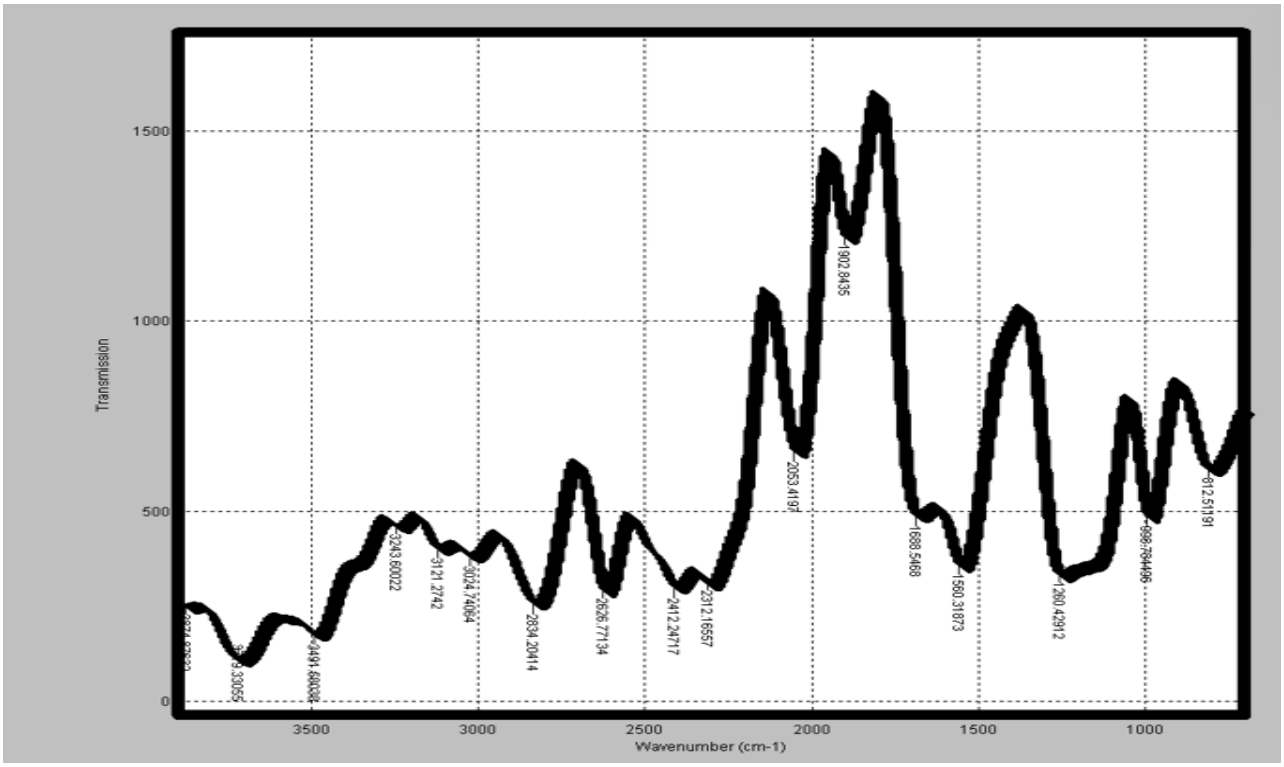

Figure 2: FTIR of ethanol extract *B. monandra*

Table 2: FTIR Interpretation of Ethanol Extract *B. monandra*

S/N	Wavelength (cm^{-1})	Functional Group	Compounds
1	812.5119	C-CI	Chloro compound
2	998.7845	C-0-C	Ether C-O stretch
3	1260.429	RNH_2	1^0 amine NH stretch
4	1560.319	H-C=CH	Vinylidene antisymmetric stretch
5	1688.547	R_2NH	2^0 amine NH stretch
6	1902.843	R-S-C≡N	Thiocyanate SCN stretch
7	2053.420	RC00H	Carboxylic acid C0 stretch
8	2312.166	$R_2C=0$	Carbonyl C=0 anti-symmetric stretch
9	2412.247	R-C≡N	Nitriles CN antisymmetric stretch
10	2626.771	CH_2	Methylene C-H symmetric stretch
11	2834.204	CH_2	Methylene C-H symmetric stretch
12	3024.741	RCH0H	1^0 alcohol 0H stretch
13	3121.274	RCH0H	1^0 alcohol 0H stretch
14	3243.600	R_3N	3^0 amine NH stretch
15	3491.580	RCH0H	1^0 alcohol 0H stretch
16	3719.331	R_3CH0H	3^0 alcohol 0H stretch
17	3874.876	R_3CH0H	3^0 alcohol 0H stretch

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4.0 DISCUSSIONS

The antiplasmodial potential of the ethanolic extract of *B. monandra* leaves was assessed using mice. *In vivo* models, such as mice, are frequently employed due to their ability to consider both prodrug effects and the likely participation of the immune system in eliminating pathogens[16]. In essence, non-primate animal models cannot be infected by *Plasmodium* species that cause disease in humans[17]. Therefore, researchers employ the rodent malaria parasite to evaluate the effectiveness of antimalarial drugs in rodents through in vivo studies, specifically relying on the susceptibility of *P. berghei* to these medications[18]. Ethanol was selected as the extracting agent for the active components in the plant leaves due to its exceptional solvent properties. This choice was supported by the indication of high total polyphenolic content and strong antioxidant activity associated with the ethanol extraction method[13].

The gradual rise in parasitemia observed in the untreated infected group throughout the study aligns with the perspective that parasitemia tends to steadily increase following infection or inoculation, leading to death in the absence of appropriate treatment[19]. However, the observed increases in parasitemia reduction among the extract-treated groups with an increase in doses of the extract correlate with the findings of Tona et al., Iwaloku et al., and Agbugui et al. [20-22], who separately reported a greater reduction in the parasitemia at higher doses.

The significant reduction in parasitemia observed in the group administered 150 mg/kg of the extract, which exceeded the effect of the standard drug (ACT) throughout the three-day treatment, indicates the considerable antimalarial properties of the plants. The study revealed that the ethanolic extract of *B. monandra* leaves exhibited antimalarial effects across all concentrations used in this study. Consequently, this marks the first report on the antiplasmodial properties of B. *monandr*a. The potential antimalarial activity observed in the plant's ethanolic extract could be attributed to its composition of bioactive elements. These include pharmacologically active phytochemicals such as saponins, tannins, flavonoids, phenols, and alkaloids, which were identified in the plant extracts (as listed in Table 1). These components have been suggested to function as primary antioxidants or scavengers of free radicals, potentially mitigating the oxidative damage caused by the malaria parasite[23-24]. It has also been reported that many kinds of metabolites, including polyphenols, diterpenoids, and quinines, are present in the crude extracts of *B. monandra* leaves [25-27]. Diterpenoids have been confirmed to have antimalarial properties[28]. Studies have shown that the antiplasmodial effect of natural products of plant origin may be mediated via inhibition of protein synthesis[29]. Moreover, polyphenols in this plant extract, which has an antioxidant effect, may also contribute to the antimalarial activity[30]. This is because the antioxidant activities of polyphenols can inhibit heme polymerization, and the unpolymerized heme is very toxic to the *Plasmodium* species. Also, quinine found in this plant has been used for the treatment of malaria for over 400 years. These phytochemicals may be acting singly or in synergy with one another to produce the observed anti-malarial activity in this study.

5.0 CONCLUSIONS

Bauhinia monandra showed a very good curative effect against established infection. This indicates that the plant could be

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considered as a new resource for generating agents that could act as alternative antimalarial drugs to overcome *Plasmodium* resistance.

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