EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 1
607-614
Year: 2019

# ANTIMALARIAL ACTIVITY OF *ALYSICARPUS ZEYHERI* (HARV), *BORRERIA SCABRA* AND *UAPACA PILOSA* (HUTCH) EXTRACTS ON MICE INFECTED WITH *PLASMODIUM BERGHEI* BERGHEI

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**Article Received on 20/11/2018** **Article Revised on 10/12/2018** **Article Accepted on 30/12/2018**

## ABSTRACT
The extracts of *Alysicarpus zeyheri(Harv)* (whole plant), *Borreria scabra* (whole plant) and *Uapaca pilosa(Hutch)* (leaves) were evaluated for their anti-malarial properties in *Plasmodium berghei* infected mice. *In vivo* curative anti-plasmodial model as well as body temperature and weight changes produced by these extracts were used as parameters for their anti-malarial potential. The phytochemical analyses of the extracts were carried out using standard protocol while the oral acute toxicity was evaluated in mice using Lorke's method. The curative anti-plasmodial activity was evaluated in mice infected with chloroquine-sensitive strain of *P. berghei* Berghei using a limit dose of 500 mg/kg for each sample. Weights and temperatures of the mice were measured periodically. The oral median lethal doses of the extracts were estimated to be greater than 5000 mg/kg body weight each. Of the three extract, *B. scabra* at 500 mg/kg produced the most significant ($p<0.05$) curative effect with 73.33% suppression on Day 1 and 100% on Day 4. Chloroquine (5 mg/kg) was more potent than the extracts in the test models. *U. pilosa* exerts a significant ($p>0.05$) temperature decrease of infected mice when compared to the negative control from day 1 to day 4. There were no significant changes in temperature by *A. zeyheri*, *B. scabra* extracts and chloroquine. No significant changes in body weight were observed at all test drugs when compared to the negative control. The results suggest that extracts of *B. scabra* showed the most curative anti-plasmodial effect in mice which may be attributed to the presence of phytochemical constituents such as alkaloids, flavonoids and terpenoids.

**KEYWORDS:** Anitimalaria, Plasmodium *berghei* Berghei, *Alysicarpus zeyheri*, *Borreria scabra* and *Uapaca pilosa extracts.*

## INTRODUCTION
Plants have always been considered to be a possible alternative and rich source of new drugs and most of the anti-malarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates.[1] *Alysicarpus zeyheri*, *Borreria scabra* and *Uapaca pilosa* are plants that are being used traditionally in the management of malaria infections in Zaria and other parts of Northern Nigeria.

The use of medicinal plants to cure diseases dates back to prehistory. They have formed the basis of Traditional Medicine (TM) practice. Plants have been relied upon to support, promote, retain and regain human health, and are used worldwide.[2][3]

Malaria is disease caused by *Plasmodium* parasites which is transmitted through bites of infected female anopheles mosquito. *Plasmodium falciparum* is the most virulent of all the five *Plasmodium* species that causes malaria infection. With high morbidity and mortality, malaria has continued to be life threatening and thus it is a danger to public health. Reports of world Health Organisation[4] estimated that 3.2 billion people worldwide are at risk of malaria infection with sub-Saharan Africa alone accounting for 13% while the global cases of malaria infection in 2015 was 212 million with 429, 000 deaths. Though there have been a slight decrease in the rate of mortality among endemic countries over the past 15 years, there are still major concerns of devastating impact on quality of life of people.

Even as they are recommended as the first line of treatment, Artemisinine based combination therapies (ACTs) still presents new challenges as growing reports of resistance in malaria endemic areas to these conventional anti-malarials emerges.[5] The several side effects caused by orthodox drugs like gastrointestinal tract (GIT) disturbance, vertigo, itching, skin rashes, nausea, vomiting, etc and a tremendous rise in cost of medicines in Africa[6], there is need for novel agents from plants that can overcome current problems of malaria therapy.

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## MATERIALS AND METHODS

### Collection and Authentication of Plants

The whole plant of *B. scabra* and *A. zeyheri* and the leaves of *U. pilosa* were obtained from Ahmadu Bello University Farms, Zaria in Kaduna State, Nigeria with the help of a traditional herbalist. The plants were authenticated by Mallam Namadi Sanusi (a taxonomist with Ahmadu Bello University) and the voucher specimen of the plant samples were deposited at the herbarium of the Department of Biology, Ahmadu Bello University, Zaria, with voucher number 2811, 791 and 965 for *B.scabra*, *A.zeyheri* and *U.pilosa* respectively.

### Extraction of Plant Materials

The whole plant of *B.scabra*, *A.zeyheri* and the leaves of *U.pilosa* were air dried at room temperature. The dried plant parts were crushed and ground into coarse powder using a clean pestle and mortar. 200 g of each plant material were dissolved in 1.2 litres (1200ml) of 96% ethanol each in a separate bottle for two weeks. The mixture was filtered with ash less filter paper, the extract was concentrated using rotary evaporator at a temperature of 40°C. The extracts were stored in a refrigerator at 4°C in well-closed containers to protect from light and moisture.

### Phytochemical Screening

Phytochemical screening were carried on the plants extracts using standard procedures, to identify the chemical constituents present as described by standard methods.[7][8][9]

### Parasite strain

Chloroquine-sensitive *P. Berghei berghei* (NK 65 strain) was sourced from the National Institute for Medical Research (NIMR), Lagos, Nigeria. The parasite was maintained in the animal facility centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja by continuous re-infection through intraperitoneal passage in mice.

### Animals

Male and female Swiss mice weighing 18 – 30 g were housed in plastic cages, fed with standard rodent chow and allowed free access to potable water at temperature ranging between 25 – 28$^{o}$C and 12 hours light/dark cycle with relative humidity in a well-ventilated room. They were allowed to acclimatize for two weeks in the animal facility centre of the Department of Pharmacology/Toxicology, NIPRD before the commencement of the work. All experiments were carried out in accordance with National Research Council (NRC) guidelines for the care and use of laboratory animals.[10]

### Acute toxicity and determination of median lethal dose ($LD_{50}$)

Oral acute toxicity study of extracts of *B. scabra*, *A. zeyheri* and *U. pilosa* were carried out in mice using the Lorke's method.[11] In the first phase, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg extract/kg body weight orally respectively. They were observed critically for four hours after dosing and then 24 hours later for death and any signs of toxicity such as writhing, decreased motor activity and respiratory depression. The second phase of the study consisted of three fresh mice, one per group, with each receiving oral doses of 1600, 2900 and 5000 mg/kg body weight, respectively based on the result of the first phase. They were also carefully observed for signs of toxicity and mortality critically for the first four hours and subsequently for 24 hours. Observations at both phases continued daily for a further 14 days. These were carried out for all three extract samples. The $LD_{50}$ was calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose of the extract, i.e. the geometric mean of the successive dose that produced 0 and 100% survival rates.

$LD_{50} = \sqrt{maximum\ non\text{-}lethal\ dose \times minimum\ lethal\ dose}$

### Parasite inoculation

Each mouse was intraperitoneally inoculated with 0.2 ml inoculum estimated to contain$1\times 10^{5}$ -$10^{7}$ *P. berghei* Berghei-infected erythrocytes. This was prepared with blood from a heavily infected donor mouse, diluted with an appropriate volume of physiological saline solution.

### Curative antiplasmodial assay

The curative activity of extracts of *B. scabra*, *A. zeyheri* and *U. pilosa* in established infection was evaluated using the method of Ryley and Peters.[12] Twenty five mice were inoculated intraperitoneally with 0.2 ml standard inoculums containing about $0.1\times10^{7}$ *P. berghei* parasitized red blood cell. On day three, the mice were randomized into test groups of five mice each (n=5). Treatments were carried out as follows: Group 1 and 2 served as positive and negative controls and received normal saline (10 ml/kg) and chloroquine (5 mg/kg b.w) respectively, while other test groups were treated with equal doses (500 mg/kg) of extracts of *A. zeyheri*, *B.*

*scabra*, and *U. pilosa* respectively. Treatment was continued once daily on days 4 to 6. On day 7, blood films were made, stained with Geimsa and the level of parasitaemia assessed. The films were then examined microscopically and parasitaemia was expressed as the mean number of parasitized erythrocytes counted in 10 fields of approximately 200 erythrocytes per field. The percentage suppression of parasitemia was calculated for each test sample by comparing the parasitemia in untreated infected controls with those of treated test sample groups and the results was multiplied by 100.

$$Percentage\ suppression = \frac{mean\ control\ parasitemia - mean\ test\ parasitemia}{mean\ control\ parasitemia} \times 100$$

**Temperature/Survival time**

The rectal temperature of each mouse used in the test for all three test samples was measured with digital thermometer by holding the mouse at an angle of 45$^o$ with its head upward. The probe was dipped into lubricant (Olive oil), inserted approximately 2cm into the rectum, and held for 20 sec until a stable temperature was obtained (a beeping sound from the thermometer).

**Statistical analysis**

Data were expressed as mean ± standard error of mean (SEM). Graph pad prism version 6.02 was used to analyze the data. The differences between means were compared using One way analysis of variance (ANOVA) followed by Dunnet's post hoc test with $p<0.05$ considered as statistically significant.

## RESULTS

**Phytochemical Screening of *Alysicarpus zeyheri*, *Borreria scabra* and *Uapaca pilosa***

Phytochemical analysis carried out on the ethanol extract of the whole plant of *A.zeyheri* showed the presence of tannins, saponins, alkaloids, flavonoids, volatile oils, phenols and terpenoids. *B.scabra*(whole plant) was found to contain tannins, saponins, alkaloids, flavonoids and terpenoids. Similarly, the leaf extract of *U.pilosa* contains tannins, saponins, alkaloids, flavonoids, glycosides, phlobatannins, cardenolides and terpenoids (Table 1).

**Table 1: Results of Phytochemical screening of the ethanol extracts of *A.zeyheri*, *B. scabra* and *U. pilosa*.**

| Phytochemical Constituent | *A.Zeyheri* | *B.scabra* | *U. pilosa* |
|---|---|---|---|
| **Alkaloids** | + | ++ | + |
| **Flavonoids** | ++ | ++ | + |
| **Glycosides** | - | - | +++ |
| **Saponins** | ++ | + | +++ |
| **Tannins** | + | + | + |
| **Phenols** | +++ | _ | _ |
| **Terpenoids** | ++ | ++ | ++ |
| **Cardenolides** | - | - | + |
| **Phlobatannins** | - | - | +++ |
| **Volatile Oils** | ++ | - | - |

+++: Highly Present; ++: Moderately Present; +: Present in trace; -: Absent.

**Oral acute toxicity**

In the oral acute toxicity tests, no mortality was recorded on *B. scabra*, *A. zeyheri* and *U. pilosa*. The $LD_{50}$ was therefore estimated to be greater than 5000 mg/kg. Other treatment-related toxicity signs observed in both phases of the study in all samples were paw licking, excessive sniffing and restlessness. No further signs of toxicity or death were seen after 14 days of observation as shown in (Table 2) below.

**Table 2: The Acute oral toxicity of the Plant Samples.**

| | Dose/Extract Mg/Kg | Mortality | |
|---|---|---|---|
| | | D/T | Latency(hr) |
| **Phase 1** | 10 | 0/3 | - |
| | 100 | 0/3 | - |
| | 1000 | 0/3 | - |
| **Phase 2** | 1600 | 0/1 | - |
| | 2900 | 0/1 | - |
| | 5000 | 0/1 | - |

D/T = Dead/ Treated mice; Latency = Time of Death.

**Curative Effect of test samples**

From the results of the three test samples of *A. zeyheri*, *B. scabra*, and *U. pilosa*, *B. scabra* produced the most effective anti-plasmodial effect. This is evident in it's significantly ($p>0.05$) time dependent parasite suppression from D0 to D4. Although the antiplasmodial activity of *B. scabra* and *U. pilosa* were significant ($p>0.05$) but were not able to suppress the parasite

growth over time. Chloroquine (5 mg/kg) exerted significant ($p< 0.05$) chemo-suppression from Day 1(D0) to Day4 (D4). (Table 3 and Fig 1).

**Table 3: Curative effect of test samples on parasite count.**

| | Normal Saline | 500 mg/kg *A. zeyheri* | 500 mg/kg *B. scabra* | 500 mg/kg *U. pilosa* | 5 mg/kg chloroquine |
|---|---|---|---|---|---|
| **D0** | 2.20±0.20 | 1.60±0.24 | 1.80±0.20 | 1.60±0.24 | 2.00±0.00 |
| **D1** | 2.40±0.24 | 1.80±0.20 | 0.60±0.24*** | 1.00±0.32*** | 0.60±0.24*** |
| **D2** | 2.60±0.24 | 2.00±0.00 | 0.40±0.24*** | 1.80±0.20 | 0.40±0.24*** |
| **D3** | 2.80±0.20 | 2.20±0.37 | 0.20±0.20*** | 2.60±0.24 | 0.60±0.24*** |
| **D4** | 3.00±0.00 | 2.20±0.37 | 0.00±0.00*** | 2.20±0.37 | 0.20±0.20*** |

* ($p< 0.05$); ** ($p< 0.01$) *** ($p< 0.001$) significantly different from the control; Values were presented as Mean ± SEM; n= 5 for each group; D: Day

Key : D0= Day 1; D1= Day 2; D2= Day 3; D3= Day 4; D4= Day 5

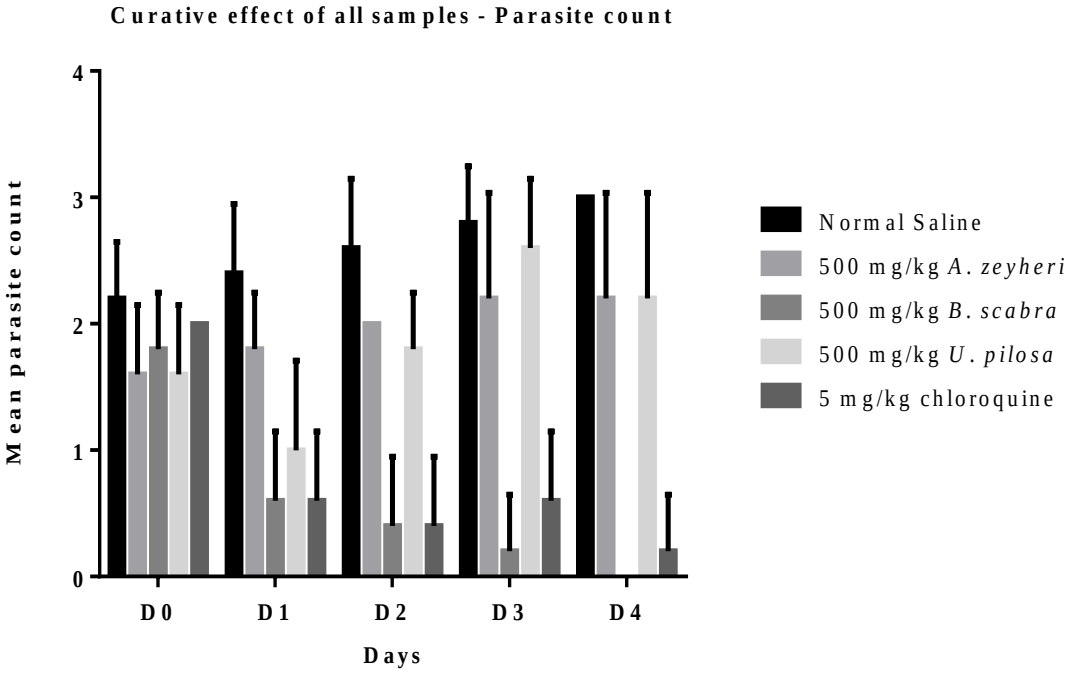



**Figure 1: Effect of test samples on mean parasite count.**

Evaluation of the suppressive activity of the plants extracts during early infection shows that the extracts produced a dose-dependent chemosuppressive effect at the various doses employed in this study (250, 500, 1000 mg/kg/day). The effects of these extracts were significant ($P<0.001$) when compared with the control as shown in (table 4) below.

**Table 4: Effect of test sample on parasite suppression.**

| | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| **Normal saline** | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| **500 mg/kg *A. zeyheri*** | 23.33±9.99 | 20.00±8.16 | 16.67±19.72 | 26.67±12.47 |
| **500 mg/kg *B. scabra*** | 73.33±11.30*** | 86.67±8.16*** | 93.33±6.67*** | 100.00±0.00*** |
| **500 mg/kg *U. pilosa*** | 60.00±11.30*** | 26.67±12.47 | 6.67±6.67 | 26.67±12.47 |
| **5 mg/kg chloroquine** | 76.66667±9.99*** | 80.00±12.25*** | 76.67±9.99*** | 93.33±6.67*** |

*** ($p< 0.001$) significantly different from the control; Values were presented as Mean ± SEM; n= 5 for each group; D: Day.

**Effects on temperature changes**

Sample C exerts a significant (p>0.05) temperature decrease of infected mice when compared to the negative control. There was no significant change in temperature by extracts of *A. zeyheri*, *B. scabra*, and 5 mg/kg chloroquine as shown in table 4. and Fig 2.

**Table 5: Curative Effect of All Samples on Temperature.**

| | Normal saline | 500 mg/kg *A. zeyheri* | 500 mg/kg *B. scabra* | 500 mg/kg *U. pilosa* | 5 mg/kg chloroquine |
|---|---|---|---|---|---|
| **D0** | 38.30±0.37 | 35.78±0.79 | 37.00±0.28 | 37.68±0.15 | 37.32±0.32 |
| **D1** | 38.26±0.22 | 35.82±0.87 | 35.94±0.66 | 35.56±0.38 | 37.66±0.21 |
| **D2** | 36.74±0.41 | 36.16±0.89 | 36.24±0.58 | 34.52±0.84** | 36.96±0.31 |
| **D3** | 36.36±0.35 | 36.46±0.88 | 36.78±0.58 | 34.08±1.07*** | 37.26±0.59 |
| **D4** | 36.10±0.15 | 36.66±0.85 | 37.08±0.58 | 33.80±1.20*** | 36.22±0.33 |

** (p< 0.01) *** (p< 0.001) significantly different from the control; Values were presented as Mean ± SEM; n= 5 for each group; D: Day

Key : D0= Day 1; D1= Day 2; D2= Day 3; D3= Day 4; D4= Day 5

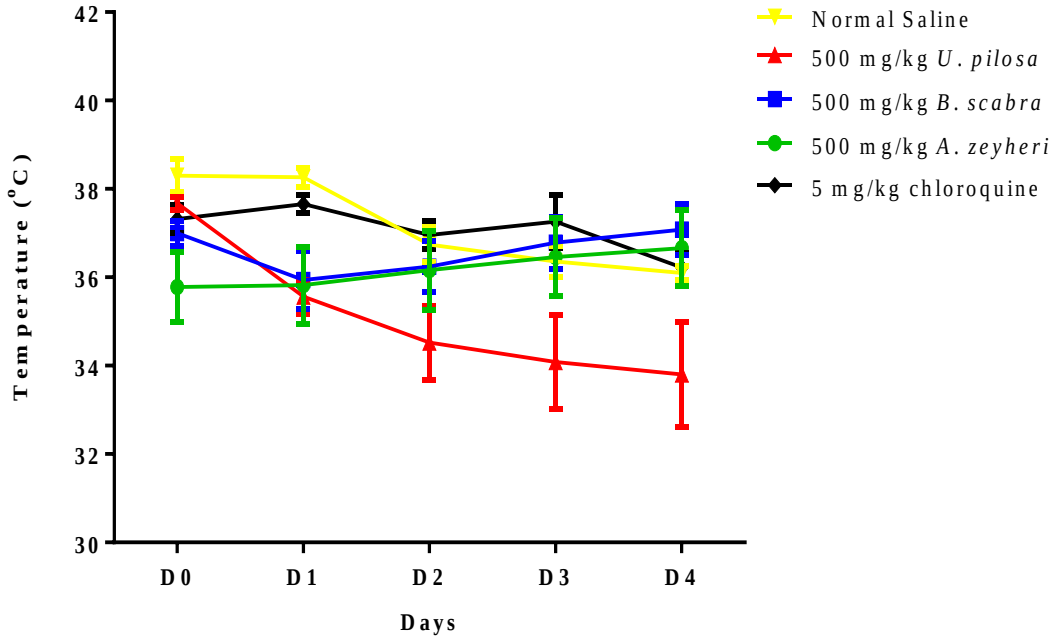



**Fig. 2: Effect of Test Samples on temperature.**

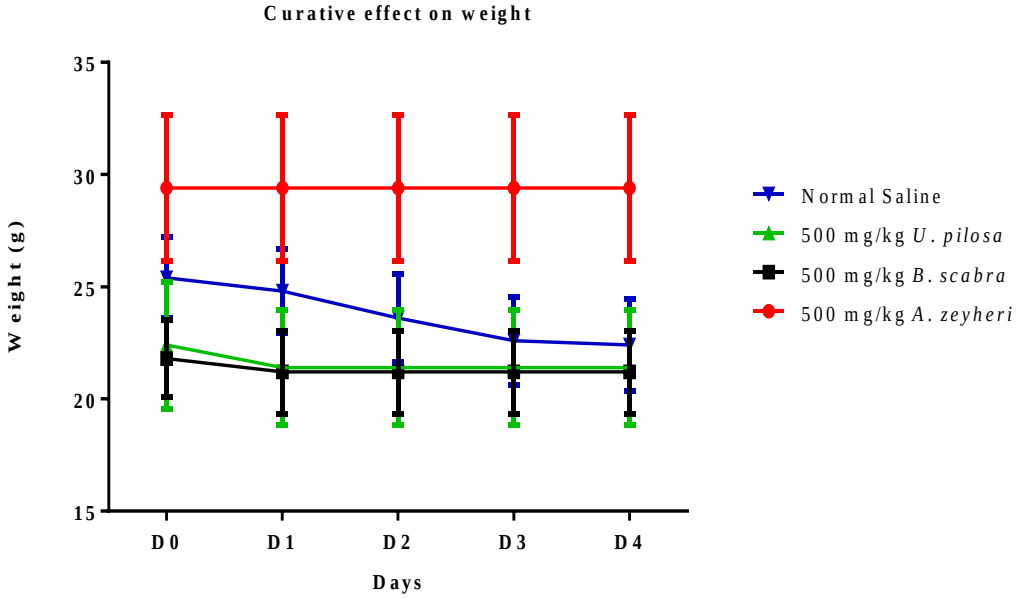
**Effects of test samples on body weight**

The effect of all three samples on changes in body weight produced no significant result when compared to the negative control even though the negative control showed a slight decrease in weight over time. (table 6).

**Table 6: Curative Effect on Weight.**

| | Normal saline | 500 mg/kg Sample A | 500 mg/kg Sample B | 500 mg/kg Sample C |
|---|---|---|---|---|
| **D0** | 24.80±1.81 | 29.40±3.23 | 21.80±1.71 | 22.40±2.84 |
| **D1** | 23.60±1.88 | 29.40±3.234 | 21.20±1.85 | 21.40±2.56 |
| **D2** | 22.60±1.96 | 29.40±3.23 | 21.20±1.85 | 21.40±2.56 |
| **D3** | 22.40±1.96 | 29.40±3.23 | 21.20±1.85 | 21.40±2.56 |
| **D4** | 25.40±2.06 | 29.40±3.23 | 21.20±1.85 | 21.40±2.56 |

No significant change when compared to Do




**DISCUSSION**

Plants have been the basis of the majority of current anti-malarial medicines. Compounds such as quinine, lapachol and artemisinin were originally isolated from herbal medicinal products. After improvement and combination with other active ingredients, they now make up the current armamentarium of medicines. In recent years, advances in screening technologies have allowed testing of millions of compounds from pharmaceutical diversity for anti-malarial activity in cellular assays and these initiatives have resulted in thousands of new sub-micromolar active compounds which are starting points for new drug discovery programmes. Natural products, especially plants based on herbal remedies are in use in the community, and have the potential unique advantage that is backed up with clinical observational data.[13]

The preliminary phytochemical tests carried out on the ethanol extract of the whole plant of *A.zeyheri* and the leaf of *U.pilosa* showed the presence of alkaloids and tannins in traces but alkaloids was moderately present in *B.scabra*, Terpenoids and flavonoids were moderately present in all the plants with the exception of *U. pilosa* that shows the presence of flavonoids and cardenolides in traces. Volatile oils were moderately present in *A.zeyheri*. Phlobatannins, saponins, glycosides and anthraquinones were highly present in the leaf of U.pilosa and phenols was also found to be highly present in *A.zeyheri*. The absence of cardenolides, glycosides, phlobatannins and anthraquinones was noticed in *A.zeyheri* and *B.scabra*. The phytochemicals present in the tested plant's parts is an indication that the plant is of pharmacological importance.[14] Compounds like flavonoïds and tannins are good antioxidant substances which have been reported to have biological activities and prevent or control oxidative stress related disorders.[15] Presence of flavonoid indicates the natural occurring phenolic compound, with beneficial effects in the human diet as antioxidants and neutralizing free radicals. Previous studies also indicated that many phenolic and steroidal compounds possessed anti-plasmodial activities.[16] These constituents have been found in other natural products which exhibited antimalarial activity.[17] Plants which contain many phytochemicals with biological activities like alkaloids and flavonoids could serve as sources of antimalarial drugs.[18]

*In vivo* studies represent the first crucial steps in research and it is paramount towards the understanding of human diseases. Mice model was used because of its many similarities with the pathway and immune responses in the annihilation of malarial infection in human.[19] *P. berghei* has been extensively used in early stages of antimalarial drug discovery and development[20][21], due to its characteristic ability to sequestrate with microcirculation similar to that in severe malaria. Young mice (2- 3 months old) were used to avoid all the possible physiological effects like anemia associated with aging in older mice which may affect the treatment outcome.[22]

This study employed the *in vivo* malarial models to evaluate anti-malarial activities as well as their effect on body temperature and weight of three extracts of *A. zeyheri, B. scabra,* and *U. pilosa* used traditionally in the management of malaria infection in Nigeria.

Phytochemical studies carried out on the plant extract showed the presence of phytochemicals that have been reported to have anti-malarial properties such as saponins, tannins and alkaloids.

In the evaluation of the anti-malaria effects of the three plant sample, a single middle dose 500 mg/kg of the intended test doses was selected and used across the plant sample for a curative study. This dose was reached from an earlier $LD_{50}$ determination which was found to be safe at tested dose of 5000 m/kg for the three extracts.

Pararsite percentage clearance is considered the most dependable parameter in the *in vivo* test.[23] A mean parasite count of ≤ 90% of the negative control group usually indicates the activities of the test in standard screening studies.[24] Extract of *B. scabra* at 500 mg/kg produced the most effective anti-plasmodial activity when compared to normal saline over a five days evaluation period with significant ($p<0.001$) and time dependent decrease in parasite count from D0 to D4 (table 1 and 2). There was complete clearance of malaria parasite on D4 of the test evaluation of *B. scabra*. *A. zeyheri* and *U. pilosa* did not produce significant suppression of parasite over the four days evaluation period. No significant changes were observed in temperature and weight differences for extracts of *A. zeyheri* and *B. scabra*. However, *U. pilosa* caused a gradual significant ($p>0.05$) decrease in core body temperature from day one to day five (D0 to D4).

The presence of the parasite alone in the blood does not induce disorder, but the response of the host immune system against foreign pathogenic organism via generation of free radicals, activation of phospholipase cascade series and generation of prostaglandins and other haemolytic principles such as free fatty acids does.[25][26] Therefore, it is possible that the phytochemicals present in extract of *B. scabra* could be having its perceived anti-plasmodial properties singly or in synergy.

## CONCLUSION

From the three test samples, extract of *B. scabra* showed a more promising anti-plasmodial sign against *P. berghei* Berghei in mice. However, more studies are required to extensively explore its anti-malarial properties. Research activities is in progress to isolate the antiplasmodial compounds from the whole plant extracts of *B.scabra.*

## AKNOWLEDGEMENTS

The authors of this work acknowledge the positive contributions of Prof Suleiman Mudi of the Department of Pure and Industrial Chemistry, Bayero University, Kano. Also, we wish to thank the research team of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Abuja ably led by the former head of the department, late Dr. Tijani Adeniyi Yahaya, may God grant him eternal rest.

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