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# Antiplasmodial Activity shown by Secondary Metabolites Extracted from the Seeds of*Pentaclethramacrophylla*Benth

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**Abstract**. Oil extracts from the African oil bean seed (*Pentaclethramacrophylla*Benth.) was analyzed for its phytochemical and mineral content and proximate, physicochemical and anti-microbial analyses were also performed. Phytochemical analysis showed the presence of tannins, saponins, quinones, terpenoids, phenols and coumarins in the oil sample. Mineral determination of the cotyledon showed the presence of iron (Fe) (with the highest concentration), Cu, Zn, Mn, Cr, Pb and Cd; while proximate analysis gave the following result: moisture (14.2%), ash content (1.5%), crude fibre (4.9%), crude proteins (12.8%), oil contents (4.9%), and carbohydrate (61.8%). GC-MS analysis of the partitioned petroleum ether and chloroform fractions of the oil revealed the presence of 9-Octadecenoic acid, 9,12-Octadecadienoic acid and their methyl esters,cis-9-Hexadecenal among the many components of the oil extract. Physicochemical analysis of the oil indicateda saponification value (148.67 mg KOH/g), peroxide value(8.0 meq/g), iodine value (10.41 mg iodine/g) and free fatty acid (8.98 mg KOH/g). The need for the development of new drugs for malaria led to our study of the antiplasmodial activity of the oil from the seeds of *Pentaclethramacrophylla*. Toxicological studies were carried out to determine the $LD_{50}$with chloroquinediphosphate as positive control and normal saline as negative control. Using the Peter's 4 day suppressive test a parasite inhibition rate of 47.72% (25 mg/kg), 63.63% (50 mg/kg) and 61.36% (100 mg/kg) on day 4 after treatment was recorded. A 95.45% chemo-suppression was observed for animals treated with 10 mg/kg chloroquine. This resultis an indication that the extract had appreciable signs of chemosuppression.

Keywords: *Pentaclethramacrophylla*, phytochemical analysis, antimicrobial analysis, GC-MS, Plasmodium, secondary metabolites, chemo-suppression, malaria

## 1. INTRODUCTION

In rural Africa, people are exposed to a variety of plants, fruits and seeds, some of which are taken as food because of their nutritional value and some for treatment of various ailments because of their medicinal properties (Opara*etal*., 2013). *Pentaclethramacrophylla*Benth. also known as oilbean tree but locally called the African oil bean tree is one such plant whose seeds are eaten by a group of people in Southeastern Nigeria and the extracts from its leaves and stem bark have been used for treatment of ailments such as diarrhea, leprosy wounds and convulsion and has been used as an anthelmintic (Fern, 2014; Akindahunsi, 2004). The seeds are gotten from perennial legume tree called *Pentaclethramacrophylla*, Benth and also commonly known as the oil bean tree. It has also been suggested that consumption of the seeds increases the hemoglobin value; increases oxygenation of tissue; enhances specific hormones and quickens the production of red blood cells (Okpala, 2015).

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Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today (Olasehinde*et al*., 2015). Based on the emergence of Plasmodium falciparum resistance to artemisinin derivatives (ART) in Cambodia, which is currently threatening the world's malaria control and elimination efforts (Arieye*t al*., 2014), development of new drugs will be a welcome initiative. There are several approaches to discovering new agents, of which discovery from natural sources is a highly desirable approach.

A high proportion of the global population still depends upon the herbal/traditional drugs for their health care. Because of their ready availability, believe in their safety and effectiveness, rural people rely on these plants for their health care issues andneeds. *Pentaclethramacrophylla*Benthis widely used as food and for treatment of some ailments and is widely abundant in Nigeria; hence this study is focused on investigating its properties and harnessing them for the treatment of malaria. The investigation was done by extracting oil from the seed cotyledon with ethanol and petroleum ether as solvents.

The oil extracted from the cotyledon and seed cover was tested for its antimicrobial and antimalarial activities. These were determined along with its chemical composition using Gas chromatography–mass spectrometry (GC-MS). The cytotoxicity and in vivo toxicity; evaluation of in vitro and in vivo antiplasmodial activities as well as the determination the LD50 and chemo-suppressive properties of ethanolic extracts against *Plasmodium bergheibergheiwere* considered.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The African oil bean (*Pentaclethramacrophylla*) seeds were obtained from Oyingbo market, EbuteMetta, Lagos, Lagos State, Nigeria.The seeds were authenticated at the Federal Research Institute of Nigeria (FRIN), Ibadan. It was assigned a voucher No: Pm/Bio/H806 with Ascension code: OgCUNo807 Thecover of the seeds of *Pentaclethramacrophylla* were manually removedwith knife and cut into small sizes of about 5 mm. These were then stored in a refrigerator until required.

### 2.2 Plant Extraction

The extraction was performed by taking a weighed amount of the seed and placing it in a thimble of the Soxhlet extractor. The extraction was performed exhaustively with the appropriate solvent (ethanol or petroleum ether).After the extraction, the solvent was removed using a rotary evaporator. The oil collected was keptin the refrigerator for further analysis. The oil percentage extract was calculated as:

$$\%\ oil\ content = \frac{weight\ of\ oil\ extracted}{weight\ of\ seed\ sample} \times 100$$

The ethanolic oil extract was partitioned between chloroform, petroleum ether and distilled water. About seventeen (17) grams of ethanolic sample extract was first partitioned between water (50 mL) and petroleum ether (50 mL) in a 250 mL separating funnel. The water fraction was then poured back into the separating funnel and 50 mL of chloroform was added to the mixture and the chloroform fraction was obtained. Both the petroleum ether and chloroform fractions were allowed to evaporate and analyzed by GC/MS.

### 2.3 Gas Chromatography/ Mass Spectroscopy

The GC-MS analysis was carried out at Shimadzu Training Center for analytical instruments (STC) Lagos. The GC-MS instrument used for the analysis was GCMS-QP2010SE SHIMADU JAPAN. The machine comprises of injector and gas chromatograph interfaced to mass spectrophotometer. The gas

chromatography condition was: column oven temperature (60$^{o}$C); injection temperature (200$^{o}$C); injection mode split ratio (1:1); helium as carrier gas flow rate (1.56 mL/min); the system was temperature programmed from 60$^{o}$C(at 10$^{o}$C/min) to 160$^{o}$C(held for 2min) then at (10$^{o}$C/min) to 250$^{o}$C; the injection volume was 0.5 µL. Mass spectrophotometer condition: Ion source temperature 200$^{o}$C; Interface Temperature 250$^{o}$C solvent cut time 4.5 min and acquisition was in the scan mode.

**2.4 Physicochemical and Proximate Analysis**

Physicochemical and proximate analyses were performed using the standard methods as described in the literature. Antimicrobial analysis was performed according to the method previously described by Olasehinde*et al*., (2016).

**2.5 In vivo Antimalarial Activity**

**2.5.1 Experimental Animals**

Experimental animals used in this study were the Swiss pre-infected albino mice (*Musmusculus*) of either sex, weighing between 18 – 25 g. All animals were housed in standard cages and kept in the animal house, at Covenant University, Ota, Ogun state, Nigeria. The animals were fed with a balanced standard pellet diet and maintained under standard laboratory conditions (24 – 28oC), standard light cycle and water ad libitum according to the NRC guide, for the care of laboratory animals(Institute for Laboratory Animal Research, 2011).They were allowed to acclimatize for five days before the commencement of the experiment. Ethical approval for use of animals was obtained from the BIOSREC, Covenant University.

**2.5.2 Parasites**

The chloroquine sensitive Plasmodium bergheiNK65 rodent parasite used to assess the antimalarial potential of *Pentaclethramacrophylla*was obtained from the National Institute for Medical research (NIMR), Lagos, Nigeria.

Each mouse was inoculated with 2 mL of plasmodium infected blood, containing about 0.5*103 of parasitized erythrocytes intra-peritoneally. This volume was prepared by determining the percentage parasitemia and the erythrocyte count of the donor mice and diluting 15 – 20 drops of blood, from the tail tip of the infected albino mice with 25 mL of Phosphate buffered solution (PBS). The animals were then left to attain maximum parasitemia for seven days.

**2.5.3 Acute Oral Toxicity**

Lorke's method was used to evaluate the acute toxicity of the ethanol extract of *Pentaclethramacrophylla*, which then allowed us todetermine the $LD_{50}$(Lorke, 1983).

Twelve mice were selected and divided into three groups of four mice each. The groups of mice were administered intra-peritoneally, with doses of 25 mg/kg, 50 mg/kg and 100 mg/kg respectively. The mice were observed for signs of toxicity, which included paw licking, salivation, stretching of the whole body, weakness, sleep, respiratory disease, coma and death in the first four hours and subsequently daily for 7 days. The animals were fasted for 24 h before the commencement of the experiment.

The number of deaths in each group within 24 hours was recorded and oral median lethal dose $LD_{50}$ was calculated using this formula:

$$LD_{50} = \sqrt{minimum\ toxic\ dose \times maximum\ tolerated\ dose}$$

**2.5.4 In Vivo Schizontocidal Activity 4-Day Peters' Test**

The Peter's 4-day suppressive test against chloroquine sensitive *Plasmodium berghei*NK65 infection in mice was employed to test for suppressive activity(Peters, 1968). Adult Swiss Albino mice weighing 20 – 25 g were inoculated by intra-peritoneal injection with standard inoculum of

*Plasmodium bergheiberghei*with 1×107 infected erythrocytes. The mice were randomly divided into 3 groups of 4 mice each for the *P. macrophylla*extract for 25 mg/kg, 50 mg/kg and 100 mg/kg. Two control groups were used; the positive control was treated daily with 10 mg of chloroquine/kg, while the negative control group was given 2 mL/kg normal saline. The body weight of each mouse was measured on the first day, and the fifth day, while the body temperature was taken before and after drug administration (D0),and monitored daily to the fifth day.

On each day of the experiment, blood was collected from the tail of each mouse and a smear was made on the microscope slide to make a film(Saidu et al., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes and parasitemiaexamined.Parasitemia was determined by Light microscopy using a 100× objective lens and the following equation was used to calculate the average percentage chemo-suppression:

$$\%\, chemosuppression = \frac{A - B}{A} \times 100$$

Where: A = Average parasitemia in the negative control group; B = average parasitemia in the test group.

Parasitemia estimation was done by allowing the slide to dry in a vertical position and examined under a light microscope at ×100 magnification and determining the number of parasites per field on a slide.

## 3. RESULT AND DISCUSSION

Percentage oil extracted from the seeds of P. macrophylla was very low; ethanol (4.89%)and petroleum ether (2.75%). This low oil content is a confirmation of the result reported by (Sofowora*et al*., 2013) but contrasts significantly with values reported by other workers(Odoemelam, 2005; Ogueke*et al*., 2010; Oyeleke*et al*., 2014) whose values were obtained in the process of fermenting the seed cotyledon.

The results of the proximate analysis, phytochemical screening and physicochemical analysis of the oil extract are presented in tables 1, 2 and 3 respectively. Table 2 shows the presence of family of compounds whose activities are well known and have been reported on many occasions(Chung *et al*., 1998; Ntie-Kang *et al*., 2014; Oyeleke*et al*., 2014).

**Table 1:**Results of proximate analysis of seed

| Proximate Analysis | Moisture | Ash Content | Crude Fiber | Proteins | Oil Content | Carbohydrate |
|---|---|---|---|---|---|---|
| Determined Values (%) | 14.16 | 1.53 | 4.85 | 12.79 | 4.89 | 61.78 |

**Table 2:** Results of phytochemical screening of seed oil

| Component | Tannins | Saponins | Quinones | Terpenoids | Phenols | Coumarins |
|---|---|---|---|---|---|---|
| Observation | + | + | + | + | + | + |

**Table 3**: Results of the physicochemical properties

| Test | Saponification value (mg-KOH/g) | Free fatty acid (FFA) (mg-KOH/g) | Peroxide value (meq/kg) | Iodine value (mg-iodine/g) | pH | |
|---|---|---|---|---|---|---|
| | 148.665 | 8.98 | 8.0 | 10.4058 | 4.57 (ethanol extract) | 4.20 (petroleum ether extract) |

**Table 4**:Resultsof Mineral composition in seed

| **Minerals** | Iron | Copper | Zinc | Manganese | Chromium | Cadmium | Lead |
|---|---|---|---|---|---|---|---|
| **Concentration (mg/L)** | 3.0271 | 1.4102 | 1.0703 | 0.4663 | 0.0568 | 0.0075 | 0.0278 |

The minerals analyzed for are as shown table 4 above. The minerals found present apart from sodium (Na), potassium (K) and calcium (Ca), which were not analyzed for are iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), cadmium (Cd) and lead (Pb). The values obtained for these minerals compare very well with those obtained byOyeleke*et al*., (2014). These minerals have all been reported as essential for the body to function properly(Dana *et al*., 2014; Ikhuoria*et al*., 2008). GC-MS analysis was carried out on the crude extract, petroleum ether and chloroform fractions to determine the components of the oil. The chromatograms of the petroleum ether and chloroform fractions are shown in Figures 1 and 2 below.

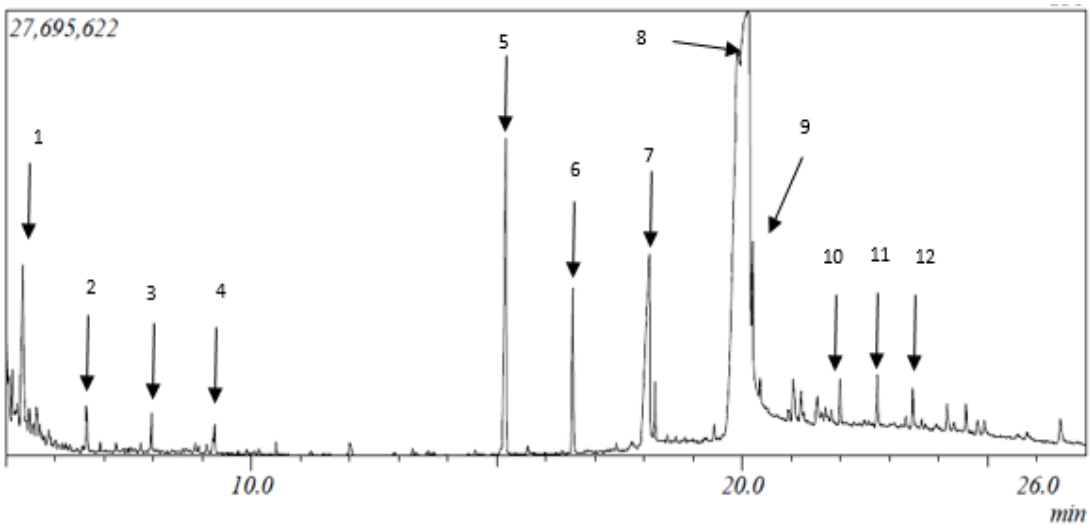


**Figure 1:**Chromatogram of the petroleum ether fraction

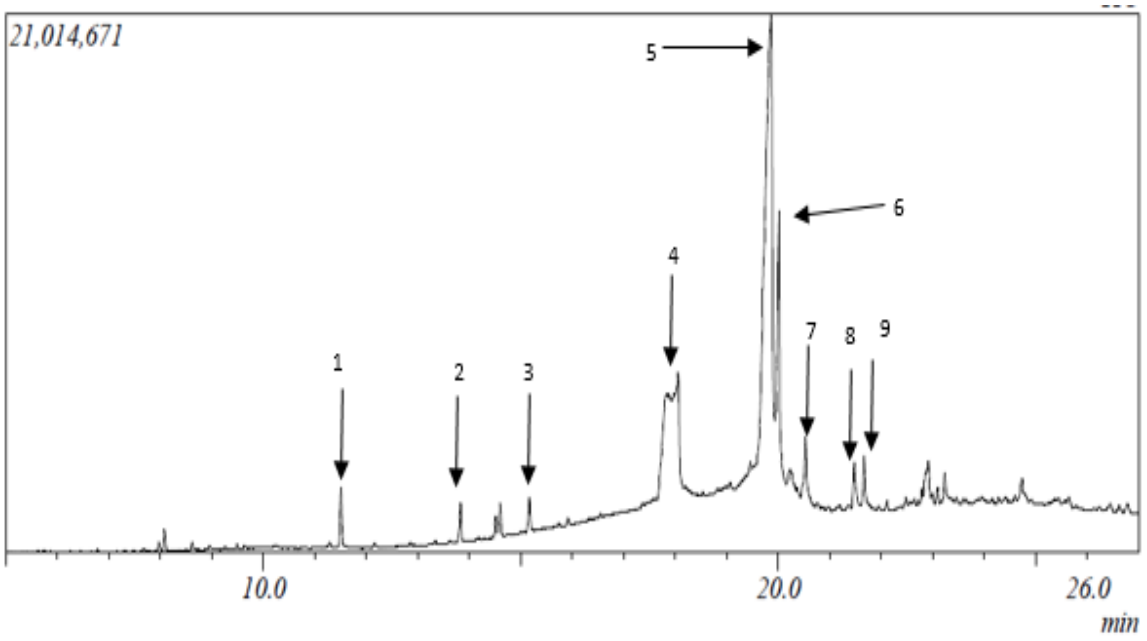


**Figure 2:**Chromatogram of the chloroform fraction

The identity of numbered peaks in the chromatograms was confirmed by comparison of their mass spectra with mass spectra of the authentic compound from literature. For the petroleum ether fraction, the following compounds were identified as numbered:**1** – Undecane (5.338); **2** – dodecane (6.648); **3** – tridecane (7.965); **4** – tetradecane (9.246); **5** – isobutyl, butyl glutaric acid (15.180); **6** – dibutyladipate (16.547); **7** – n-hexadecanoic acid (18.104); **8** – 9,12-octadecadienoic acid (19.985); **9** – Octadecanoic acid (20.205); **10** – 2-methylhexacosane (21.988); **11** – pentatricontane (22.744).

For the chloroform fraction, the following compounds were identified as numbered: **1** – Vanillin (11.510); **2** – 5-oxo-Pyrrolidinecarboxylic acid-ethyl ester(13.829); **3** – 1-(1-cyclohexen-1-yl)-Pyrrolidine (15.165); **4** – n-Hexadecanoic acid (17.834); **5** – 9-Octadecenoic acid (19.857);**6** – octadecanoic acid (20.014);**7** – 9,12-Octadecadienoic acid (20.525); **8** – 2-hydroxy cyclopentadecanone (21.477); **9** – Eicosanoic acid (21.665). The number in parentheses after the name of each compound is the retention time for that compound in minutes.

The anti-plasmodial activity of petroleum ether and ethanolic extracts of seeds of *Pentaclethramacrophylla*is presented in tables 4, 5 and 6. It confirmed that petroleum ether seed extracts of *Pentaclethramacrophylla*is non-toxic as high as 100 mg/kg, Table 4.

**Table 5:** Acute toxicity test forEthanol and petroleum ether extract of seeds of *Pentaclethramacrophylla*

| Group | Average Weight (g) | Dose | Survival at 24 h | Survival at 48 h | Survival at 72 h |
|---|---|---|---|---|---|
| Group 1 | 26 g | 25 mg/kg | Active and healthy | | |
| Group 2 | 25 g | 50 mg/kg | | | |
| Group 3 | 25 g | 100 mg/kg | | | |

Table 5 represents test organisms treated with petroleum ether extract of *P. macrophylla*against *Plasmodium berghei*. The test subjects were treated with 25 mg/kg, 50 mg/kg and 100 mg/kg as indicated. From the table, it can be deduced that parasitemia decreased significantly over the test period, and no deaths were recorded.

Parasitemia in the negative control (Table 5)did not decrease but rather showed a significant increase as is characteristic of malaria. The positive control however showed a significant decrease in parasitemia.

Table 6shows the percentage chemo-suppression over time for the petroleum ether extract of *P. macrophylla*. The table shows percentage chemo-suppression of parasites at different concentrations for different days. In the table chloroquine showed the highest chemosuppression rates followed by the 100mg/kg of the petroleum extract.

**Table 6**: Mean Daily Parasitemia at Various Concentrations ofEthanol Extract of *P. macrophylla*

| Concentration (mg/Kg) | Mean Percentage Parasitemia | | | | |
|---|---|---|---|---|---|
| | Pre-Treatment (%) | Day 0 (%) | Day 1 (%) | Day 2 (%) | Day 3 (%) |
| 25 | 11.00 ±1.00 | 9.00±1.00 | 7.50±0.5 | 5.00±0.0 | 3.50±0.05 |
| 50 | 11.00±1.00 | 8.50±0.5 | 6.00±0.0 | 4.50±0.5 | 2.50±.050 |
| 100 | 13.00±1.00 | 10.50±0.5 | 8.00±0.0 | 5.50±0.5 | 2.00±0.00 |
| Chloroquine (Positive Control) | 15.00±0.00 | 10.00±0.00 | 6.50±0.5 | 4.00±1.0 | 1.00±1.00 |
| Normal Saline | 12.00±1.00 | 15.00±1.00 | 17.50±1.5 | 19.00±1.0 | 22.00±0.00 |

Values are expressed as mean ±SEM

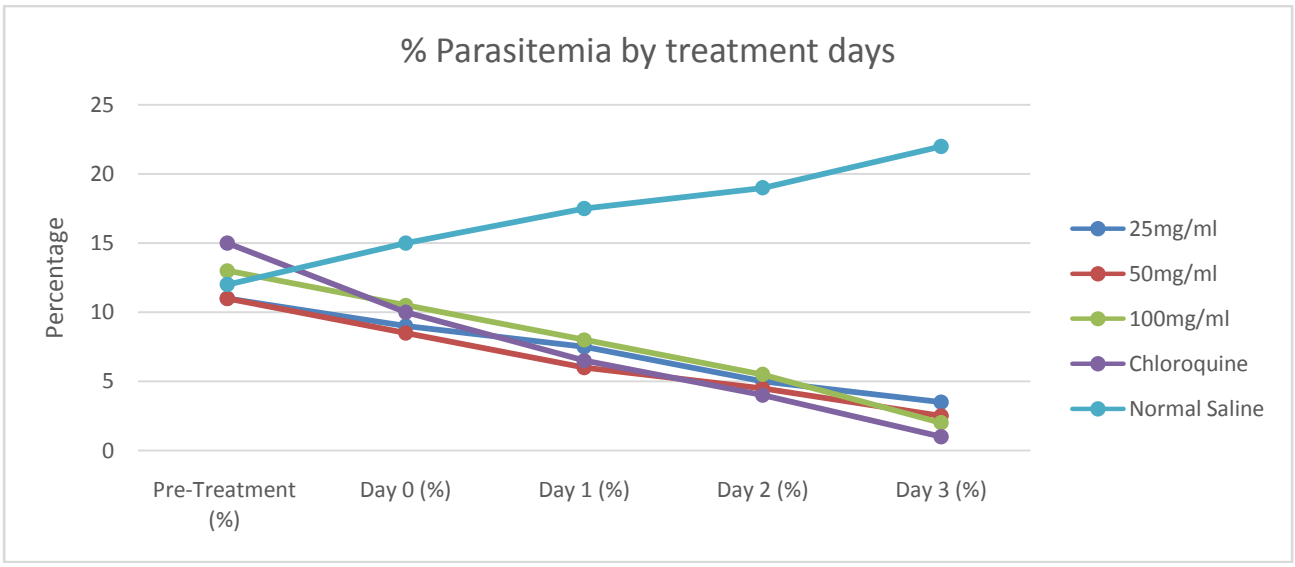


**Figure 3:** Percentage parasitemiaat Different Concentrations of Ethanol Extract of *P. macrophylla*

**Table 7**: Mean Daily Parasitemia at Various Concentrations of Petroleum Ether Extract of *P. macrophylla*

| Concentration (mg/Kg) | Mean Percentage Parasitemia | | | | |
|---|---|---|---|---|---|
| | Pre-Treatment (%) | Day 0 (%) | Day 1 (%) | Day 2 (%) | Day 3 (%) |
| 25 | 22.5 ±3.89 | 17.0±0.71 | 13.5±1.06 | 14.0±0.35 | 11.5±0.35 |
| 50 | 19.00±0.71 | 15.50±0.35 | 15.0±0.71 | 10.00±0.00 | 8.0±0.00 |
| 100 | 19.50±0.35 | 17.00±0.00 | 11.5±2.47 | 17.00±5.66 | 8.50±1.06 |
| Chloroquine (Positive Control) | 15.00±0.00 | 10.00±0.00 | 6.5±0.35 | 4.00±0.71 | 1.00±0.71 |
| Normal Saline | 12.00±0.71 | 15.00±0.71 | 17.5±1.06 | 19.0±0.71 | 22.00±0.00 |

Values are expressed as mean ±SEM

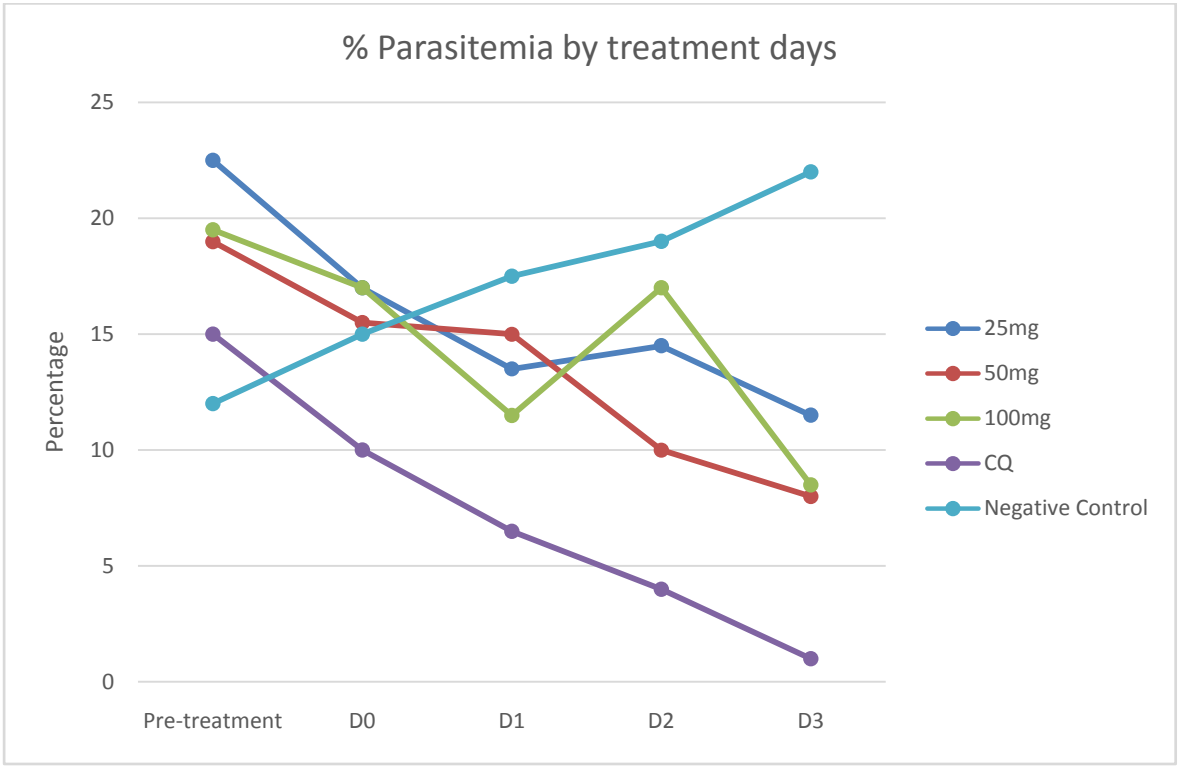


**Figure 4:** Percentage parasitemia at Different Concentrations of Petroleum Ether Extract of *P. macrophylla*

**Table 8**: Percentage Chemo-suppression of the Ethanoland Petroleum Ether extracts of *P.macrophylla*seed

| Drug | Dose | Percentage chemo-suppression (%) Ethanol | Petroleum Ether |
|---|---|---|---|
| Physiological saline | 0.5mL | - | - |
| *P.macrophylla* | 25mL/kg | 84.09 | 47.72 |
| | 50mL/kg | 88.63 | 63.64 |
| | 100mL/kg | 90.90 | 61.36 |
| Chloroquine Phosphate | 10mg/kg | 95.45 | 95.45 |

**Discussion**

The *in-vivo* antiplasmodial activities of crude ethanolic and petroleum ether extracts of seeds of *P.macrophylla*Benth of the family *Fabacaea*with voucher No: Pm/Bio/H806 and Ascension code: OgCUNo807 were determined in this study. In vivo models are usually employed in anti-malaria studies because they take into account, the possible pro drug effect and probable involvement of the immune system in eradication of the pathogen(Waako *et al*., 2005). During early infections, Peter's 4 day suppressive test was used to evaluate schizontocidalactivity. In all methods, determination of percent of inhibition of the parasitemia was the most reliable parameter. A mean parasitemia level that is ≤90% of that of the vehicle treated animals (negative control), usually indicates that the test compound is active(Peter and Anatoli, 1998). In the 4 day suppressive test, seed extracts ofP.macrophyllasignificantly reduced parasitemia in animal models in a dose dependent manner, with these drugs showing anti-malarial activities compared to that of the standard drug tested. There were no deaths or any signs of toxicity observed after oral administration of single doses up to 100mg/kg of the extracts of P. macrophylla. This data indicates that the medium lethal dose ( $LD_{50}$) should be 100mg/kg for both extracts, for test animals. There were no significant changes in the behavior, cutaneous breathing of both male and female mice.

The $LD_{50}$was calculated to be 707.11mg/kg for Petroleum ether seed extract of P. macrophylla. The $LD_{50}$is the concentration at which 50% of the test subjects will die. This shows that the extract becomes toxic at 707.11mg/kg. Also for P.macrophylla, the $LD_{50}$ was 707.11mg/kg

The seed extract of P.macrophylla exerted dose dependent chemo-suppressive effect against Plasmodium bergeibergei. The petroleum ether extract caused a 47.72%, 63.64% and 61.36% chemo-suppressions at concentrations 25mg/kg, 50mg/kg and 100mg/kg respectively by the 4th day of the test, while the standard drug chloroquine caused chemo-suppressions of 95.45%. The ethanolic extracts of P.macrophyllaseed at dosing concentrations of 25mL/kg, 50mL/kg and 100mL/kg body weight of mice yielded 84.09%, 88.63% and 90.90% inhibition respectively as against 95.45% for the standard drug, chloroquine. The observed higher efficacy of Chloroquine may in part be due to non-selectivity of the extract or slow absorption and poor bio-availability of the extract. This is common with medicinal plant extracts(Adzu and Haruna, 2007).

P. Bergeibergei has been utilized in studying the activity of potential anti-plasmodials in-vivo in rodents (Pedroni *et al*., 2006)and it produces diseases similar to those of human plasmodial infection (Kumar et al., 2008). Agents with suppressive activity against P. Bergeibergeiwere known for their anti-plasmodial activity(Elufioye and Agbedahunsi, 2004).

## 4. CONCLUSION

This study has shown the efficacy of *P. macrophylla*as an antimalarial and as such validates its use in traditional settings. Future research should focus on identifying the active fractions of the plant.

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