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Antimalarial Activity of Ethanol Extract of *Mucuna pruriens* Leaves on Nk65 Chloroquine Sensitive Strain of *Plasmodium berghei*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OEE supervised the study and drafting of the protocol. Author OVA managed the analyses of the study. Author FMI performed the statistical analysis and wrote the first draft of the manuscript.

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ABSTRACT

Mucuna pruriens leaves are used in some part of Nigeria for the treatment of malaria and anemia. With an estimated 3.3 billion people in 97 countries and territories at risk of being infected with malaria according to the WHO, researching into new chemotherapeutic agent against this disease is indeed necessary. This study was designed to evaluate the antimalarial effect of ethanol extract of *Mucuna pruriens* leaves on NK65 chloroquine sensitive strain of *plasmodium berghei* in mice. The bioactive compounds in the extract were identified using GC-MS. The experimental animals were divided into 6 groups: negative control, normal control, groups treated with chloroquine (10 mg/kg), Artemeter/Lumefantrine-ACT (20 mg/120 mg/kg), 500 mg/kg of *M*. pruriens, 1000 mg/kg of *M*. pruriens and 2000 mg/kg of *M*. pruriens respectively. Parasite inoculation was done by intraperitoneal injection of 0.2ml of the inoculum (1×10^7 infected erythrocytes). The GCMS result revealed the extract contains n-hexadeconoic acid, a compound known to possess antimalarial

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properties. The study revealed that the administrations *M. pruriens* leaves extracts at suitable doses reduced the parasite load and were able to maintain the PCV at a normal range with a stabilising effect on body weight.

Keywords: Antimalarial; parasitemia; Mucuna pruriens; bioactive compounds; alternative medicine.

ABBREVIATIONS

ACT : Artemisinin Combination Therapy
CQ : Chloroquine
GC-MS : Gas Chromatography Mass Spectroscopy
PCV : Packed Cell Volume
WHO : World Health Organisation

1. INTRODUCTION

Malaria is a life-threatening disease prevalent in most tropical and subtropical regions, according to World Health Organisation (WHO), an estimated 3.3 billion people in 97 countries and territories are at risk of being infected with malaria, and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). The burden is very heavy in the WHO African Region, where an estimated 90% of all malaria deaths occur with children aged under 5 years, accounting for 78% of all the deaths [1].

Medicinal plants are discovered and utilized throughout human history. Plants have the capability to synthesize wide range and varieties of phytochemical compounds that have the ability to exert important biological functions. Some of these phytochemicals have been widely researched and confirmed to possess medicinal properties.

Mucuna pruriens; a plant belonging to the family Fabaceae, an annual climbing legume of relative height of about 3-18 meter, which is indigenous to tropical regions, especially Africa, India, and the West Indies. Its leaves possess the potential to act as a booster for red blood cell (RBC) production [2], in the process of treatment it is believed to revert the anemia resulting from malaria.

This research is designed to explore the antiplasmodial potentials of the ethanol extract of *Mucuna pruriens* leaves against chloroquine sensitive strain of *Plasmodium berghei.*

2. MATERIALS AND METHODS

2.1 Plant Collection

Mucuna pruriens leaves were collected from a farm land in Ejule, Ofu Local Government of Kogi State, Nigeria and identified in the Department of Biological Sciences, Kogi State University Anyibga, Kogi State.

2.2 Experimental Animals and Malaria Parasite

The mice with average weight of 25 g were purchased from the animal section of Salem University, Lokoja, Kogi State. The animals were acclimatized, in the experimental room for 2 weeks.

Mice infected with chloroquine sensitive NK 65 *Plasmodium berghei* (1×10^7 infected red blood cells) were gotten from the Institute of Advanced Medical Research and Trainings (IAMRAT), College of Medicine, University of Ibadan, Nigeria.

2.3 Preparation and Extraction of Plant Sample

The leaves of *Mucuna pruriens* leaves were air dried and ground into powdery form using a Binatone BLG-450 blender. The ground leave samples were extracted with Soxhlet extractor, using absolute ethanol as solvent.

The extracts were dried in evaporating dishes after solvent recovery in the Soxhlet extractor, the extracts were transferred to an oven for further drying at the temperature of 40^0C. The percentage yield was also calculated by subtracting the weight after extracting from the weight before extraction.

2.4 Gas Chromatography Mass Spectrophotometry (GC-MS)

GC-MS analysis of the ethanol extract of *M. pruriens* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS)

equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 25:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 60 °C (isothermal for 2 min), with an increase of 30 °C/min to 120°C, ending with a 3 min isothermal at 290 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 700 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 21 min.

2.5 Inoculation of Malaria Parasite

The donor mice were anesthetized chloroform, blood was taken from them by cardiac puncture and immediately transferred into normal saline. The experimental animals were infected by intraperitoneal injection of 0.2ml of the inoculum (1×10^7 infected erythrocytes), they were then left for 4 days to allow spread and multiplication of the parasite in their blood according to the method of [3].

2.6 Experimental Design

The inoculated mice with average weight of 25 g were grouped as follows into minimum of 3 animals per group with average weight of 25 g:

Group 1: (negative) were given only water.
Group 2: (normal control) not inoculated with the parasite were given only water.
Group 3: (positive control 1) which were given chloroquine (10 mg/kg).
Group 4: (positive control 2) were given Artemeter/Lumefantrine (ACT) 20 mg/120 mg/kg.
Group 5: were given 500 mg/kg of *M*ucuna pruriens.
Group 6: were given 1000 mg/kg of *M*ucuna pruriens.
Group 7: were given 2000 mg/kg of *M*ucuna pruriens.

2.7 Treatment of Experimental Animals

The experimental animals were treated accordingly for 3 days following the details of their grouping above. The administration of the drugs and extract was done orally.

2.8 Weight Determination

The experimental animals were weighed before parasite inoculation, after parasite inoculation, before and after treatment.

2.9 Determination of Packed Cell Volume (PCV)

The packed cell volume determination was done for the experimental animals after inoculation, before and after treatment [4]. This was done to deduce the effect of the parasite on the red blood cells (RBC) and also the effect of the extracts on the RBC. The PCV was done by collecting blood from the tail of the experimental animals into heparinized capillary tubes which were sealed up from one end and then centrifuged at 3000rpm for 5 minutes, the PCV was then read using a hematocrit reader.

2.10 Determination of Percentage Parasitemia

This was done according to the method by Monica [5]. Blood was collected from the tails of the experimental animals on slides and smeared into thin film. The slides were air dried and the stained with Leishman stain, this was done by adding 8 drops of the stain to the slides and leaving them for 2 minutes, the stain was then diluted with buffered water (pH 6.8) and allowed to stay for 8 minutes after which it was washed off the slides with distilled water. The slides were air dried and viewed using light microscope at × 100 magnification with oil immersion.

The parasitemia was determined before, during and after treatment.

The percentage parasitemia was calculated using a formula [3]:

> Percentage parasitemia = Number of parasitized RBC / Total number of RBC) × 100

3. RESULTS

3.1 Body Weight of Chloroquine Sensitive Strain Induced Animals Treated With *M. pruriens*

Fig. 2 shows the effect of ethanol extract of *M. pruriens* leaves extracts on body weight of chloroquine sensitive strain *Plasmodium berghei*

induced mice. There was a decrease in the body weight of the untreated group at week 2 and week 3 and these were statistically significant ($p<0.05$). There were no significant decreases or increase in the body weight of the other groups.

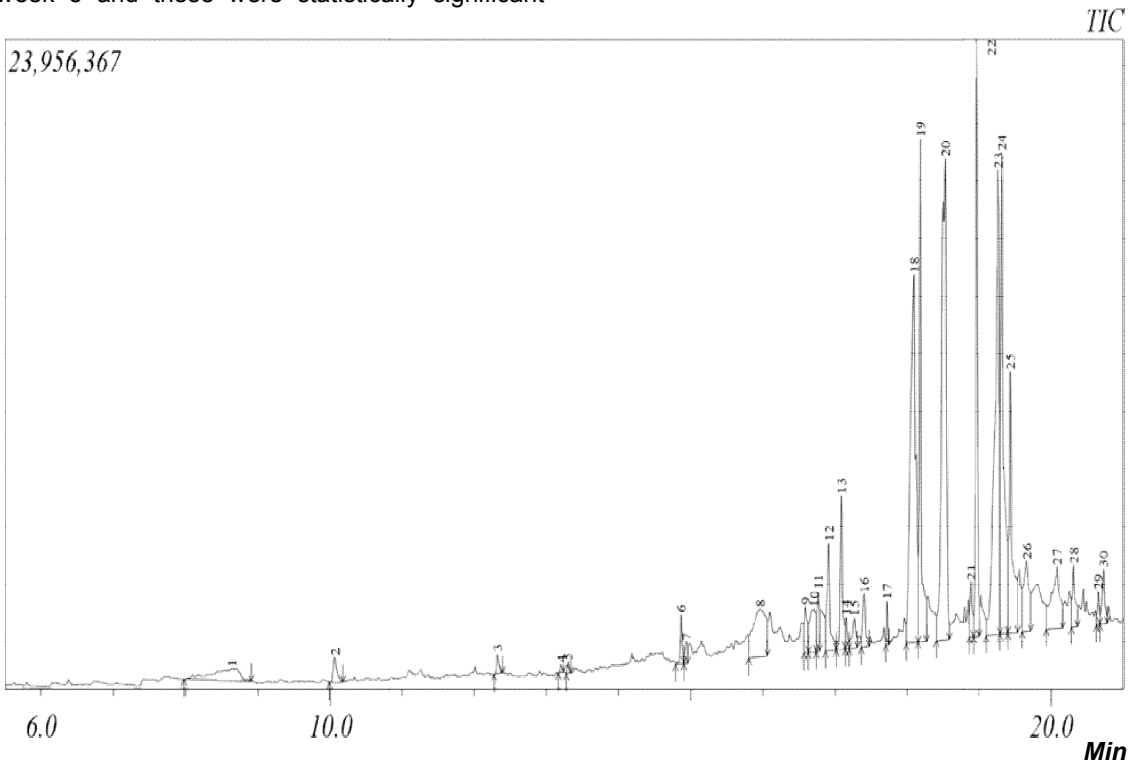

Fig. 1. Spectra GC-MS analysis of *M. pruriens*

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.640	7.975	8.908	11528695	2.19	430533	0.27	26.78	MI	Glycerin
2	10.061	9.983	10.175	3199887	0.61	883383	0.56	3.62	V	4H-Pyran-4-one,2,3-dihydro - 3,5-dihydroxy
3	12.325	12.275	12.392	1581456	0.30	732997	0.47	2.16	MI	2-Methoxy-4-vinylphenol
4	13.204	13.167	13.242	554208	0.11	300063	0.19	1.85	MI	2-Cyclohexen-1-ol, 3-methyl-6-(1-methyleth
5	13.303	13.275	13.342	655033	0.12	402868	0.26	1.63	MI	2H-Indeno[1,2-b]furan-2 one, 3,3a,
6	14.867	14.792	14.908	3304646	0.63	1851567	1.18	1.78	V	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4
7	14.941	14.908	14.967	2167251	0.41	786905	0.50	2.75	V	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
8	15.964	15.808	16.067	22157326	4.21	1769196	1.13	12.52	V	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
9	16.589	16.567	16.633	5273237	1.00	1699156	1.08	3.10	V	Tridecanoic acid
10	16.708	16.633	16.742	9263069	1.76	1583355	1.01	5.85	V	3-O-Methyl-d-glucose
11	16.769	16.742	16.792	5368234	1.02	2230724	1.42	2.41	V	3-O-Methyl-d-glucose
12	16.913	16.867	17.017	12086016	2.29	3963302	2.52	3.05	V	1-Cyclohexene-1-methanol, .alpha.,2,6,6-tet
13	17.093	17.017	17.142	13778395	2.61	5651682	3.60	2.44	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	17.150	17.142	17.200	2166124	0.41	1190982	0.76	1.82	V	Oxirane, hexadecyl-
15	17.269	17.200	17.308	3797283	0.72	1116955	0.71	3.40	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
16	17.404	17.367	17.475	5386302	1.02	1964913	1.25	2.74	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	17.721	17.700	17.750	2124329	0.40	1641386	1.04	1.29	V	Cyclopentanetridecanoic acid, methyl ester
18	18.096	17.983	18.150	69517553	13.19	13570678	8.64	5.12	V	n-Hexadecanoic acid
19	18.182	18.150	18.267	36371820	6.90	18547259	11.81	1.96	V	Hexadecanoic acid, ethyl ester
20	18.530	18.408	18.583	81127466	15.40	17749909	11.30	4.57	V	Squalene
21	18.896	18.867	18.933	5188852	0.98	2063883	1.31	2.51	V	9,12,15-Octadecatrienoic acid, 2,3-dihydrox
22	18.977	18.933	19.008	35049289	6.65	22013835	14.01	1.59	V	Phytol
23	19.268	19.108	19.292	67308650	12.77	17125959	10.90	3.93	V	Ethanol, 2-(9,12-

										octadecadienyloxy)-, (Z,Z)
24	19.318	19.292	19.408	54229443	10.29	17740232	11.29	3.06	V	9,12,15-Octadecatrienoic acid, ethyl ester, (
25	19.435	19.408	19.542	27968391	5.31	9634051	6.13	2.90	V	Hexadecanoic acid, ethyl ester
26	19.662	19.600	19.717	13634200	2.59	2604192	1.66	5.24	V	7-Hexadecenal, (Z)-
27	20.084	19.933	20.158	17866577	3.39	2285775	1.45	7.82	V	1-Heptatriacotanol
28	20.308	20.275	20.367	6749611	1.28	2294504	1.46	2.94	V	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
29	20.653	20.625	20.675	2257462	0.43	1230171	0.78	1.84	V	Nonanoic acid, 9-oxo-, ethyl ester
30	20.720	20.675	20.775	5243736	1.00	2043798	1.30	2.57	V	1-Heptatriacotanol

3.2 Packed Cell Volume (PCV) of Chloroquine Sensitive Strain Induced Animals

Table 1 shows the effect of ethanol extract of *Mucuna pruriens* leaves on packed cell volume (PCV) of chloroquine sensitive strain *Plasmodium berghei* induced mice. There was a decrease in the PCV of the untreated animals and those treated with CQ, but these decrease were not statistically significant at ($p>0.05$), likewise there was increase in the PCV of the group treated with ACT and those treated with 500 mg/kg, 1000 mg/kg, 2000 mg/kg of *M. pruriens* respectively.

3.3 Percentage Parasitemia of Sensitive Strain Induced Animals Treated with *M. pruriens*

Fig. 3 shows the effect of ethanol extract of *M. pruriens* leaves on parasitemia of chloroquine sensitive strain *Plasmodium berghei* induced mice. There was an increase in the percentage parasitemia of the untreated group, but was only significant ($p<0.05$) at day 21. There were decrease in the percentage parasitemia of the group treated with CQ which was significant at day 7, 14, and 21. There was a continuous significant ($p<0.05$) decrease in percentage parasitemia in the group treated with ACT. There was a continuous significant decrease in percentage parasitemia of the group treated with 500 mg/kg of *M. pruriens*. Also there was a continuous significant decrease in percentage parasitemia of the group treated with 1000 mg/kg of *M. pruriens*.

4. DISCUSSION

Malaria reduces the number red blood cells (RBC) in the host blood leading to an anaemic condition, this is due to low production and increased destruction of red blood cells during malaria infection [6]. It was observed in this study as shown in Table 1 that *P. berghei* chloroquine sensitive strain infected animals treated with *M. pruriens* had increase or relative stability in their PCV at doses of 500 mg/kg. This finding agrees with what was reported by Akindele and Busayo [2] on the ability of *M. pruriens* in stabilising PCV.

Reduction in body weight is one of the effects of malaria infection as the disease may lead to loss of appetite and disruption of other vital activities. In this study (Fig. 2) it was observed that there was a continuous decrease in the body weight of the untreated animals which became statistically significant at $p<0.05$ on day 14 and 21 of the infection but this was not so in the group treated with the extracts as no significant decrease in body weight was observed in them. These observations are in accordance with that of Basir and others [7].

Percentage parasitemia expresses the level of infection in the host blood, in the case of malaria parasites this is said to be the number of infected RBCs against that of normal RBCs.

This study shows (Fig. 3) that in the *P. berghei* chloroquine sensitive strain infected mice, there was an increase in the percentage parasitemia of the untreated group but was only significant at day 21 of the infection. There was a significant decrease in the percentage parasitemia of the group treated with chloroquine at day 7, 14 and 21, this is due to the fact that the parasite is sensitive to chloroquine and thus can be used to treat this particular infection, this is similar to what was observed by Odeghe and others in 2012 [8]. A significant continuous decrease in percentage parasitemia was observed in the group treated with ACT which is due to the effectiveness of ACT on malaria infection [9]. There was a continuous significant ($p<0.05$) decrease in percentage parasitemia of the group treated with the extract.

The result (Fig. 1) gotten GC-MS analysis revealed that the extract contains n-hexadeconoic acid, a compound known possess antimalarial properties amongst other bioactive compounds found.

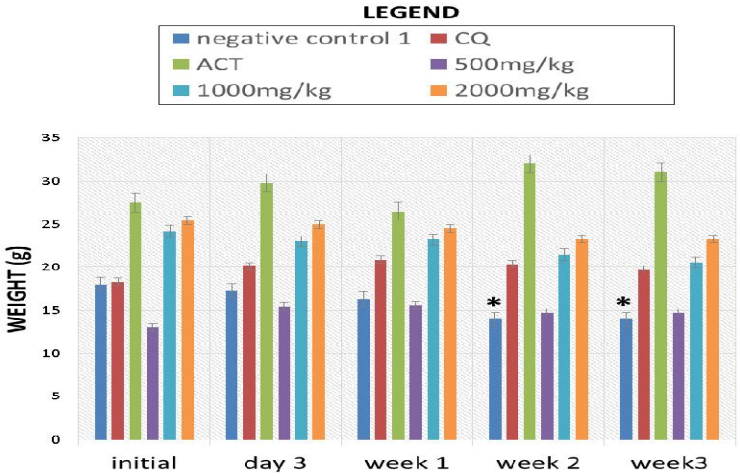

Fig. 2. Effect of ethanol extract of *Mucuna pruriens* leaves on body weight of chloroquine sensitive strain *Plasmodium berghei* induced mice

'' represents significant difference at p<0.05 when the body weight at day 3, week 1, week 2 and week 3 was compared to the initial body weight.*

Table 1. Effect of ethanol extract of *Mucuna pruriens* leaves on packed cell volume (PCV) of chloroquine sensitive strain *Plasmodium berghei* induced mice

	PCV levels (%)	
Groups and doses	**Before treatment**	**After treatment**
Negative control	41.67 ± 2.89	36.67 ± 2.89
CQ	50.00 ± 5.00	46.67 ± 5.77
ACT	41.67 ± 2.89	50.00 ± 2.00
500mg/kg (MP)	41.67 ± 2.89	50.00 ± 2.52
1000mg/kg (MP)	40.00 ± 5.00	45.00 ± 5.00
2000mg/kg (MP)	48.33 ± 7.64	50.00 ± 5.00

Values represents mean ± S.D of n=5

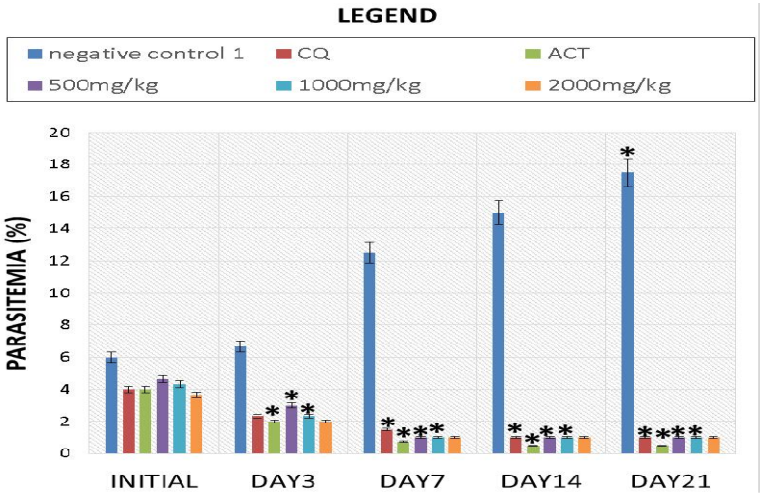

Fig. 3. Effect of ethanol extract of *Mucuna pruriens* leaves on parasitemia of chloroquine sensitive strain *Plasmodium berghei* induced mice

'' represents significant difference at p<0.05 when the parasitemia (%) of the test and control groups at day 3, day 7, day 14 and day 21 was compared to the parasitemia (%) before treatment (initial)*

5. CONCLUSION

The study revealed that the administrations of ethanol extract of *M. pruriens* leaves extracts at suitable doses reduced the parasite load and were able to maintain the PCV of the infected mice at a normal range with a stabilising effect on body weight. Since *Mucuna pruriens* are widely distributed across Africa and Asia, where malaria tends to be at its peak, further studies into the feasibility of isolating, identifying and characterising the active compounds responsible for the antiplasmodial activity of these leaves will help in reducing the malaria burden in this region.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are not publicly available due to the fact that a larger part of the whole research is undergoing further study but are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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