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Effect of *Ochna Kibbiensis* leaves on *Plasmodium berghei*

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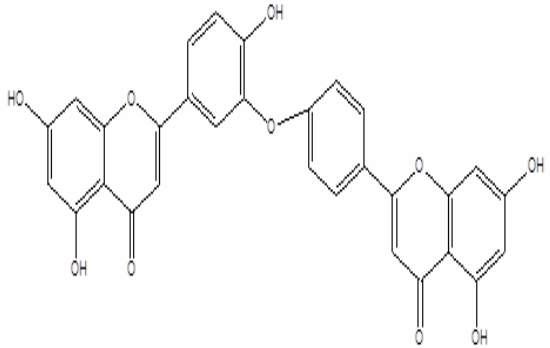
Introduction

- *Ochna kibbiensis* belongs to the Ochnaceae family
- It is a shrub or small tree found in tropical Africa from Guinea to southern and northern Nigeria

✓ **Ethnomedicinal Uses**
- ❖ Malaria
- ❖ Pain
- ❖ Asthma
- ❖ Dysentery
- ❖ Inflammation

✓ **Pharmacological actions**
- ❖ Antimicrobial (Abdullahi *et al*., 2015)
- ❖ Anti-proliferative (Abdullahi *et al*., 2016)

✓ **Phytochemistry**
- ❖ Ochnaflavone (Abdullahi *et al*., 2016)



Ochnaflavone

Picture of Ochna kibbiensis

Statement of Research Problem

Malaria is a major public health problem affecting not less than 40 % of the world's population (Snow *et al*., 2005).

An estimated 1.2 billion are at high risk of transmission (≥1 case per 1000 population), more than half of which live in the African regions and Nigeria alone accounts for a quarter of all malaria cases in Africa (WHO, 2008).

African region accounts for 81 % and 91 % of the world cases and deaths respectively, with 86 % of the mortalities observed among the under-fives (WHO, 2014).

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The disease poses severe impediment to social and economic development through a variety of ways including school and work absenteeism, high treatment expenses and decreased productivity in farming.

About 70% of Nigerians are poor and majority live in rural areas. The dare economic situation in Nigeria and many other under-developed countries necessarily means that majority of the populace cannot afford the current artemisinin-based combination therapy (ACT), where available (Fred and Ghee, 2011).

Justification of the Study

- Malaria affects the world poorest countries and largely offers unattractive market for international pharmaceutical investors (Fred and Ghee, 2011).
- *Ochna kibbiensis* is used in ethnomedicine to treat malaria (Burkill, 1985)

Aim and Objectives

❖ The aim of the study was to evaluate the effect of methanol leaf extract of *O. kibbiensis* and its n-hexane, dichloromethane, ethylacetate, and n-butanol – soluble fractions against *Plasmodium berghei*.

❖ **The work was achieved through the implementation of the following series of major activities/objectives:**

✓ The median lethal dose (LD_{50}) of the methanol extract and its fractions was determined according to Lorke's method

✓ The antimalarial effect of the extract and its fractions was investigated according to the methods described by Ryley and Peters suppressive, curative and prophylactic test using Chloroquine-sensitive *Plasmodium berghei* (NK65).

Research Hypothesis

❖ **The leaves of *Ochna kibbiensis* has antimalarial activity**

Statistical analysis

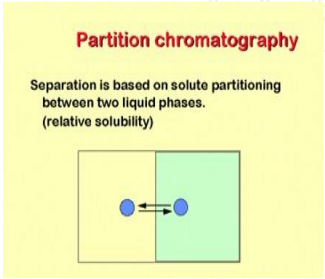
❖ Results were expressed as mean ± standard error of mean (SEM). Statistical analysis of control and test data was based on simple one-way ANOVA and Dunnett's post hoc test was used for different doses within a group. Bonferroni post hoc test was used to compare results between the means at the significance level considered at $p<0.05$

Methodology

Pulverized Leaves of O. kibbiensis

Extraction – Maceration

Crude methanol leaf extract



- **n-Hexane**
- **DCM**
- **Ethylacetate**
- **n-Butanol**

Lorke (1983)

Antimalarial assay
Ryley and Peters
- Suppressive
- Curative
- Prophylactic test
- Using Chloroquine-sensitive *Plasmodium berghei* (NK65)

Results and Discussion

Extraction and Fractionation Yield

Table 1: Percentage Yield of *O. kibbiensis*

Extract/Fraction	Weight (g)	% Yield
Methanol	825.00	22.11
n-Hexane	5.59	1.74
Dichloromethane	2.17	0.28
Ethylacetate	34.69	4.51
n-Butanol	129.29	16.79

Acute toxicity studies of the extract & fractions of *O. kibbiensis*

Table 2: Median lethal dose (LD_{50}) of extract/fractions of *O. kibbiensis*

Extract/Fraction	LD_{50} value (mg/kg)
Methanol	> 5000.00
n-Hexane	> 5000.00
Dichloromethane	> 5000.00
Ethylacetate	> 5000.00
n-Butanol	1702.94

Antimalarial studies – Suppressive test

Table 3: Suppressive effect of MEL of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Chemo-Suppression
1	NS 0.2 mL	10.00 ± 0.55	-
2	MEL 500	6.75 ± 0.75***	32.50
3	MEL 250	6.00 ± 0.71***	40.00
4	MEL 125	3.80 ± 1.02***	62.00
5	CQ 5	0.60 ± 0.24	94.00

Table 4: Suppressive effect of HF of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Chemo-Suppression
1	NS 0.2 mL	10.00 ± 0.55	-
2	HF 500	0.75 ± 0.75*	92.50
3	HF 250	1.00 ± 0.41*	90.00
4	HF 125	0.75 ± 0.48*	92.50
5	CQ 5	0.60 ± 0.24	94.00

Table 5: Suppressive effect of DCM fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Chemo-Suppression
1	NS 0.2 mL	10.00 ± 0.55	-
2	DCM 500	8.50 ± 0.65**	15.00
3	DCM 250	3.20 ± 1.46*	68.00
4	DCM 125	1.00 ± 0.00*	90.00
5	CQ 5	0.60 ± 0.24	94.00

Table 6: Suppressive effect of EF of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Chemo-Suppression
1	NS 0.2 mL	10.00 ± 0.55	-
2	EF 500	3.60 ± 0.81*	64.00
3	EF 250	3.20 ± 0.97*	68.00
4	EF 125	2.80 ± 0.86*	72.00
5	CQ 5	0.60 ± 0.24	94.00

Table 7: Suppressive effect of BF of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Chemo-Suppression
1	NS 0.2 mL	10.00 ± 0.55	-
2	BF 500	0.60 ± 0.40*	94.00
3	BF 250	0.20 ± 0.20*	98.00
4	BF 125	1.60 ± 1.17*	84.00
5	CQ 5	0.60 ± 0.24	94.00

Key: NS=Normal saline; MEL= Methanol leaf extract of OK; HF=n-Hexane fraction; DCM=Dichloromethane fraction; EF=Ethylacetate fraction; BF=n-Butanol fraction; CQ= Chloroquine; OK= *Ochna kibbiensis*. Values were expressed as Mean±SEM (n=5). Values of the group with * are statistically significantly ($p<0.05$) different from NS treated. Values of the group with superscript **are statistically significantly different from chloroquine treated groups. Values of the group with superscript ***are statistically significantly different from NS and chloroquine treated groups

Antimalarial studies – Curative test

Table 9: Curative effect of MEL of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Cure
1	NS 0.2 mL	8.60 ± 1.69	-
2	MEL 500	2.40 ± 0.87*	72.10
3	MEL 250	5.80 ± 1.56	32.60
4	MEL 125	6.40 ± 0.98	25.60
5	CQ 5	2.60 ± 0.51	69.80

Table 10: Curative effect of HF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Cure
1	NS 0.2 mL	8.60 ± 1.69	-
2	HF 500	5.00 ± 1.30	41.90
3	HF 250	7.40 ± 0.50**	14.00
4	HF 125	8.40 ± 0.68**	2.30
5	CQ 5	2.60 ± 0.51	69.80

Table 11: Curative effect of DCM fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Cure
1	NS 0.2 mL	8.60 ± 1.69	-
2	DCM 500	4.75 ± 1.25	44.80
3	DCM 250	1.00 ± 0.41*	88.40
4	DCM 125	3.40 ± 1.72	60.50
5	CQ 5	2.60 ± 0.51	69.80

Table 12: Curative effect of EF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Cure
1	NS 0.2 mL	8.60 ± 1.69	-
2	EF 500	6.00 ± 1.34	30.20
3	EF 250	4.20 ± 0.49	51.20
4	EF 125	6.25 ± 0.63	27.30
5	CQ 5	2.60 ± 0.51	69.80

Table 13: Curative effect of BF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Cure
1	NS 0.2 mL	8.60 ± 1.69	-
3	BF 500	3.60 ± 1.12*	58.14
2	BF 250	2.20 ± 1.28*	74.42
4	BF 125	0.50 ± 0.29*	94.18
5	CQ 5	2.60 ± 0.51	69.80

Key: NS=Normal saline; MEL= Methanol leaf extract of OK; HF=n-Hexane fraction; DCM=Dichloromethane fraction; EF=Ethylacetate fraction; BF=n-Butanol fraction; CQ= Chloroquine; OK= *Ochna kibbiensis*. Values were expressed as Mean±SEM (n=5). Values of the group with * are statistically significantly ($p<0.05$) different from NS treated. Values of the group with superscript **are statistically significantly different from chloroquine treated groups. Values of the group with superscript ***are statistically significantly different from NS and chloroquine treated groups

Antimalarial studies – Prophylactic test

Table 14: Prophylactic effect of MEL of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Prophylaxis
1	NS 0.2 mL	8.40 ± 0.93	-
2	MEL 500	0.20± 0.20*	97.62
3	MEL 250	3.25 ± 0.48*	61.31
4	MEL 125	2.20 ± 0.80*	73.81
5	PM 5	0.40 ± 0.24	95.24

Table 15: Prophylactic effect of HF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Prophylaxis
1	NS 0.2 mL	8.40 ± 0.93	-
2	HF 500	2.20 ± 1.02*	73.81
3	HF 250	5.40 ± 1.57**	35.71
4	HF 125	1.20 ± 0.58*	85.71
5	PM 5	0.40 ± 0.24	95.24

Table 16: Prophylactic effect of DCM fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Prophylaxis
1	NS 0.2 mL	8.40 ± 0.93	-
2	DCM 500	4.40 ± 1.63***	47.62
3	DCM 250	1.25 ± 0.95*	85.12
4	DCM 125	0.00 ± 0.00*	100.00
5	PM 5	0.40 ± 0.24	95.24

Table 17: Prophylactic effect of EF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Prophylaxis
1	NS 0.2 mL	8.40 ± 0.93	-
2	EF 500	1.75 ± 0.63*	79.17
3	EF 250	1.75 ± 1.03*	79.17
4	EF 125	0.80 ± 0.58*	90.48
5	PM 5	0.40 ± 0.24	95.24

Table 18: Curative effect of BF fraction of *OK* against *P. berghei berghei* infection in mice

S/N	Treatment (mg/kg/day)	Average Parasitaemia± SEM	% Prophylaxis
1	NS 0.2 mL	8.40 ± 0.93	-
2	BF 500	3.00 ± 1.73*	64.29
3	BF 250	2.00 ± 0.91*	76.19
4	BF 125	2.00 ± 0.77*	76.19
5	PM 5	0.40 ± 0.24	95.24

Key: NS=Normal saline; MEL= Methanol leaf extract of OK; HF=n-Hexane fraction; DCM=Dichloromethane fraction; EF=Ethylacetate fraction; BF=n-Butanol fraction; PM= Pyrimethamine; OK= *Ochna kibbiensis*. Values were expressed as Mean±SEM (n=5). Values of the group with * are statistically significantly ($p<0.05$) different from NS treated. Values of the group with superscript **are statistically significantly different from chloroquine treated groups. Values of the group with superscript ***are statistically significantly different from NS and chloroquine treated groups

Conclusion

- The findings of this study indicated that;

- The leaves of the plant *O. kibbiensis* were found to be relatively safe based on the LD_{50} values.
- The plant have demonstrated significant ($p<0.05$) antimalarial activity with suppressive, curative and prophylactic effects.
- In addition, isolation and characterization of the bioactive constituents from the most active fractions is ongoing in our laboratory.

Some references

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