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In vitro anti-plasmodial and *in vivo* malaria chemoprotective properties of methanol extract of *Zanthoxylum zanthoxyloides* root bark and its fractions

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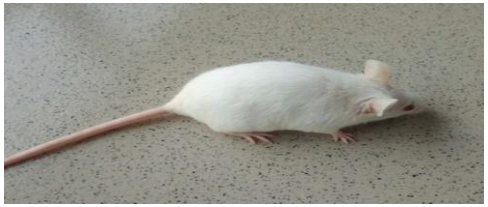
Plasmodium berghei Anka-65



- Hyper-parasitemia
- Anaemia
- Leukocytopenia
- Thrombocytopenia

Zanthoxylum zanthoxyloides root bark

Methanol

Crude methanol extract (ME)

n-hexane (NHF), ethyl acetate (EAF) and n-butanol (NBF) fractions

Inhibited *Plasmodium falciparum* (K1)

Abstract: Rural dwellers in Nigeria and other parts of West Africa use root barks of *Zanthoxylum zanthoxyloides* L. for treating sickle cell anaemia, cancers, fever and malaria. This study evaluated *in vitro* anti-plasmodial, and *in vivo* malaria chemoprotective attributes of methanol extract of *Z. zanthoxyloides* root bark (ME) and its n-hexane (NHF), ethyl acetate (EAF) and n-butanol (NBF) fractions. The *in vitro* anti-plasmodial activities against drug-resistant strain (K1) of *Plasmodium falciparum* and malaria chemoprotective properties against chloroquine-sensitive *P. berghei* in mice by ME and its fractions were evaluated using standard methods. EAF gave the highest inhibitory activities (IC_{50} = 2.76 mg/ml) against chloroquine-resistant strain (K1) of *P. falciparum,* followed by ME (IC_{50} = 5.54 mg/ml), NBF (IC_{50} =10.96 mg/ml) and NHF (IC_{50} = 11.47 mg/ml). Compared to malaria-infected-untreated mice, pre-treatment with 400 mg/kg each of EAF and ME suppressed malaria parasite growth by 92% and 81%, respectively relative to 86% by 28 mg/kg chloroquine phosphate. ME and EAF pre-treatment further prevented malaria associated cytopenia unlike infected-untreated mice. These anti-plasmodial and malaria chemoprotective properties could be attributed to phytochemicals in the herbal drugs whose mechanisms of action should be determined in further studies.

Keywords: Anti-plasmodial activity; malaria; malaria chemoprotection; *Zanthoxylum zanthoxyloides* L.

Introduction

Malaria, a notorious disease caused by *Plasmodium* species, has continued to exert great challenge to global public health, resulting in over two hundred million of hospital visits and hundreds of thousands of deaths annually (WHO, 2017). In spite of the good range of antimalarial drugs available in the market, there is still need for additional drugs; to accommodate individual pharmacogenetics preferences, and address rising pattern of drug resistance as well as the need for cheaper but effective antimalarial drugs. In many parts of Africa, decoctions of bark of *Zanthoxylum zanthoxyloides* roots are used in treating fever and malaria. This study evaluated the anti-plasmodial activities of methanol extract of the plant's root bark (ME) and its n-hexane (NHF), ethyl acetate (EAF) and n-butanol (NBF) fractions against drug resistant strains of *Plasmodium falciparum in vitro*. The study also evaluated the prophylactic effects of the ME and its EAF using *P. berghei* Anka 65-infected mice.

Results and discussion

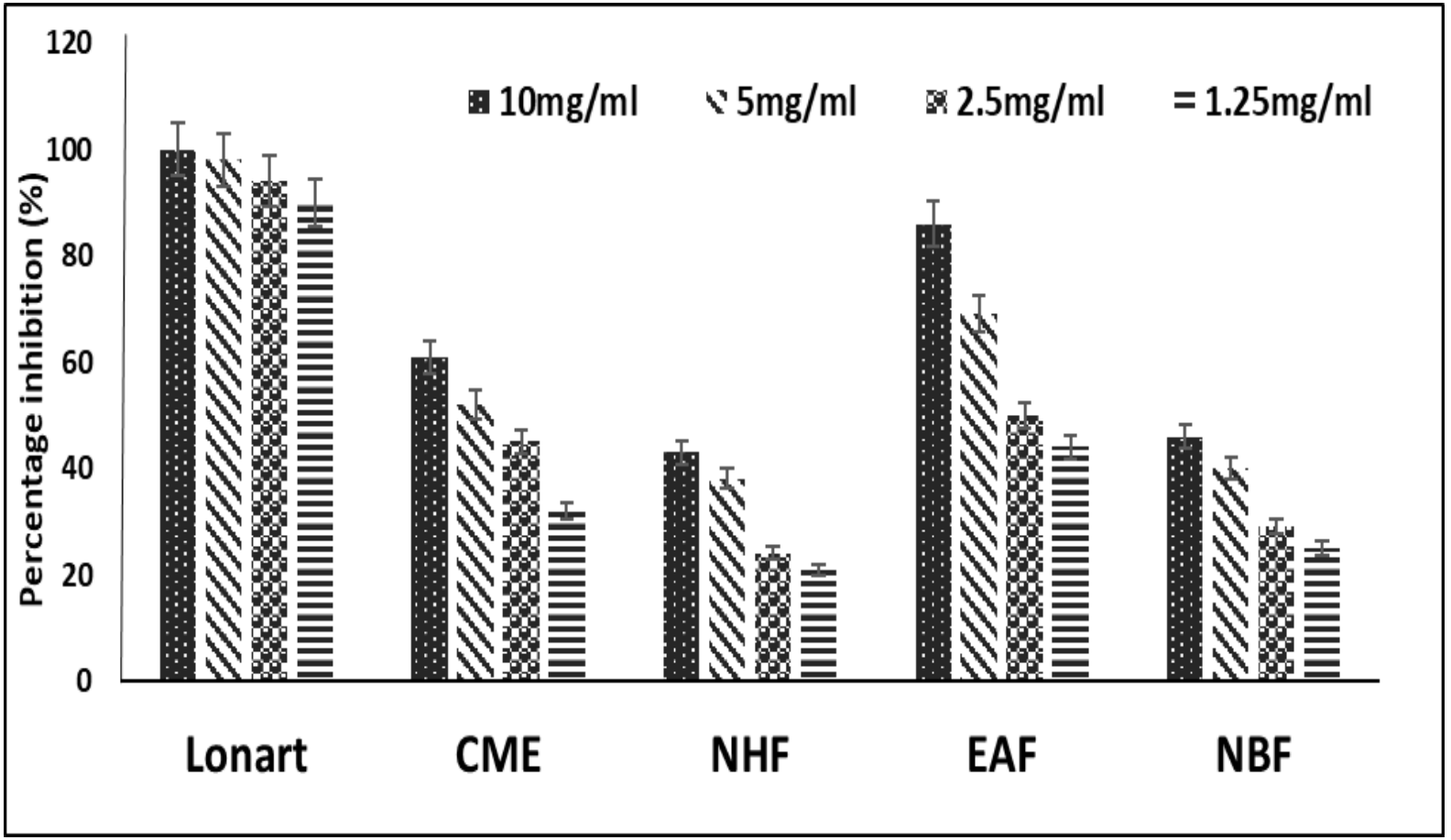


Figure 1: *In vitro* anti-plasmodial activities of ME and its N-hexane (NHF), ethyl acetate (EAF) and N-butanol (NBF) fractions

Table 1: Effects of ME and EAF on haematological parameters of malaria infected mice

Groups	Parasite suppression (%)	WBC ($x10^9$/L)	RBC ($x10^{12}$/L)	PCV (%)	Platelets ($x10^9$/L)
Normal control	0.00 ± 0.00^a	5.95 ± 0.33^f	5.95 ± 0.13^g	51.75 ± 2.36^e	243.25 ± 2.36^g
Malaria only (disease control)	0.00 ± 0.00^a	3.22 ± 0.01^a	3.35 ± 0.13^a	33.00 ± 1.41^a	167.50 ± 2.08^a
28 mg/kg b.w chloroquine + Malaria	86.11 ± 3.75^e	5.46 ± 0.17^e	5.43 ± 0.10^e	46.00 ± 1.83^d	232.50 ± 2.89^e
100 mg/kg b.w ME + Malaria	44.44 ± 3.21^b	3.47 ± 0.06^b	3.70 ± 0.12^b	36.50 ± 1.29^b	184.50 ± 4.20^b
200 mg/kg b.w ME + Malaria	63.89 ± 1.71^c	3.88 ± 0.06^c	4.23 ± 0.15^c	42.00 ± 0.82^c	201.50 ± 2.65^c
400 mg/kg b.w ME + Malaria	81.11 ± 4.22^e	4.56 ± 0.25^d	5.73 ± 0.05^f	45.75 ± 0.96^d	213.00 ± 2.45^d
100 mg/kg b.w EAF + Malaria	71.67 ± 1.67^d	4.07 ± 0.07^c	4.25 ± 0.06^c	42.00 ± 0.82^c	199.50 ± 4.11^c
200 mg/kg b.w EAF + Malaria	80.00 ± 2.28^e	5.38 ± 0.13^e	4.98 ± 0.24^d	45.50 ± 1.29^c	215.25 ± 4.11^d
400 mg/kg b.w EAF + Malaria	92.22 ± 1.91^f	6.69 ± 0.19^g	5.75 ± 0.13^f	51.50 ± 1.91^e	238.25 ± 2.36^f

Data presented above represent mean ± standard deviation; Values bearing different letters of the alphabets as superscript are significantly different at $p < 0.05$. Methanol extract of *Zanthoxylum zanthoxyloides* root bark (ME) and its ethyl acetate fraction (EAF)

Conclusions

In summary, this study demonstrated that methanol extract of *Zanthoxylum zanthoxyloides* root-bark (ME) possesses anti-plasmodial and malaria chemoprotective properties, and prevented malaria-induced hematological alterations in malaria prophylactic test.

Notably, ethyl acetate fraction of ME showed highest *in vitro* anti-plasmodial and *in vivo* malaria chemoprotective activities against chloroquine-sensitive strain of *P. berghei* compared to n-hexane and n-butanol fractions. In addition, the ethyl acetate fraction suppressed malaria-induced haematological alterations in prophylactic tests. These anti-plasmodial and antimalarial activities could be attributed to the presence of phytochemicals that are beneficial in malaria prevention.

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